High-rate anaerobic digestion of ozonated biosolids at low mesophilic temperature in a single-stage treatment process

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April 2020

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy

This thesis is dedicated to my parents

for their continued support and unconditional love,

my beloved husband,

and the new member of our family, Aiden.

TABLE OF CONTENTS

HIGH-RATE ANAEROBIC DIGESTION OF OZONATED BIOSOLIDS AT LOW MESOPHILIC TEMPERATURE IN A SINGLE-STAGE TREATMENT PROCESS I

TABLE OF CONTENTS III

LIST OF TABLES VIII

LIST OF FIGURES XI

LIST OF ABBREVIATIONS AND SYMBOLS XX

ABSTRACT XXV

RÉSUMÉ XXVII

ACKNOWLEDGMENTS XXX

PREFACE XXXI

CHAPTER 1 1

INTRODUCTION 1

1.1 INTRODUCTION 2

1.2 PROBLEM STATEMENT AND HYPOTHESES 5

1.3 RESEARCH OBJECTIVES 5

1.4 THESIS ORGANIZATION 6

1.5 CONTRIBUTION TO ORIGINAL KNOWLEDGE 8

1.6 REFERENCES 9

CHAPTER 2 12

LITERATURE REVIEW 12

2.1 BIOSOLIDS MANAGEMENT 13

2.2 ANAEROBIC DIGESTION 14

2.3 CONVENTIONAL OPERATION OF ANAEROBIC DIGESTERS AND ITS LIMITATION 16

2.4 PRE-TREATMENT METHODS 19

2.5 OZONE AS A PRE-TREATMENT METHOD 20

2.6 ANAEROBIC DIGESTION MODELS AND SYSTEM DESIGN *22*
2.7 ENERGY BALANCE AND CARBON FOOTPRINT *24*
2.7.1 Energy balance analysis 25
2.7.2 Plant-wide modeling 25
2.7.3 Carbon footprint 26
2.8 REFERENCES *28*

CHAPTER 3 **36**

ASSESSING ENERGY BENEFITS OF OPERATING ANAEROBIC DIGESTERS AT LOW TEMPERATURE WITH SOLIDS PRE-OZONATION 36
3.1 INTRODUCTION *37*
3.2 MATERIALS AND METHODS *39*
3.2.1 Reactor operation 39
3.2.2 Ozone treatment 41
3.2.3 Sampling and analytical methods 41
3.2.4 Energy balance analysis 42
3.2.5 Statistical analysis 44
3.3 RESULTS AND DISCUSSION *45*
3.3.1 Biosolids reduction 45
3.3.2 Biogas production 47
3.3.3 Soluble components 49
3.3.4 Energy balance 52
3.4 CONCLUSIONS *54*
3.5 ACKNOWLEDGEMENT *55*
3.6 SUPPLEMENTARY INFORMATION *56*
3.7 REFERENCES *59*

CHAPTER 4 **63**

EFFECT OF SLUDGE OZONATION ON KINETICS AND BIOGAS RECOVERY IN BATCH-FED AND SEMI-CONTINUOUS ANAEROBIC DIGESTION SYSTEMS AT LOW MESOPHILIC TEMPERATURE 63
4.1 INTRODUCTION *64*
4.2 MATERIALS AND METHODS *66*

4.2.1 Inoculum and substrate 66
4.2.2 Ozone treatment 66
4.2.3 Batch system - Biochemical methane potential (BMP) 67
4.2.4 Semi-continuous system – Benchtop bottle digesters 67
4.2.5 Analytical methods 68
4.2.6 Kinetic model evaluation 68
4.2.7 Microbial community analyses 69
4.3 RESULTS AND DISCUSSION 70
4.3.1 Methane yield in batch AD 70
4.3.2 Methane production in semi-continuous AD and microbial community structure analysis 73
4.4 CONCLUSIONS 80
4.5 ACKNOWLEDGEMENTS 80
4.6 SUPPLEMENTARY INFORMATION 82
4.7 REFERENCES 88

CHAPTER 5 **93**

MAXIMIZATION OF ENERGY RECOVERY AND REDUCTION OF BIOSOLIDS PRODUCTION BY COMBINING OZONATION TREATMENT AND ANAEROBIC DIGESTION AT LOW MESOPHILIC TEMPERATURE 93

5.1 INTRODUCTION 94
5.2 MATERIALS AND METHODS 95
5.2.1 Reactor operation 96
5.2.2 Ozone treatment 98
5.2.3 Sampling and analytical methods 98
5.2.4 Microbial community analyses 99
5.2.5 Statistical analysis 100
5.3 RESULTS AND DISCUSSION 100
5.3.1 Optimum ozone dose 100
5.3.2 Digestion performances in function of SRTs 101
5.3.3 Effect of SRT-HRT decoupling 104
5.3.4 Microbial community analysis 106

5.3.4.1 Bacterial and archaeal diversity 106
5.3.4.2 Taxonomic composition of metagenomes 108
5.3.4.3 Microbial dynamics across reactors 108
5.4 CONCLUSIONS *114*
5.5 ACKNOWLEDGEMENTS *114*
5.6 SUPPORTING INFORMATION *116*
Microbial community analyses 116
Bioinformatic analysis 117
5.7 REFERENCES *130*

CHAPTER 6 **135**

PLANT-WIDE MODELING OF ANAEROBIC DIGESTION COMBINING SLUDGE OZONATION AT LOW MESOPHILIC TEMPERATURE: EXPLORING ENERGY, CARBON FOOTPRINT AND COST BENEFITS 135
6.1 INTRODUCTION *136*
6.2 MATERIALS AND METHODS *138*
6.2.1 Description of WRRF under study 139
6.2.2 Plant-wide model development and calibration 139
6.2.2.1 Plant configuration 139
6.2.2.2 Model calibration and evaluation 140
6.2.3 Modeling impacts on energy and carbon footprint 142
6.2.3.1 Model configurations 142
6.2.3.2 Impact on plant energy efficiency 143
6.2.3.3 Impact on plant carbon footprint analyses 144
6.2.3.4 Impact on plant direct GHG emissions 145
6.2.3.5 Impact on plant indirect GHG emissions 146
6.2.3.6 Impact on plant cost benefits 146
6.2.3.7 Conventional vs proposed biogas system configuration 147
6.3 RESULTS *147*
6.3.1 Calibration 147
6.3.2 Plant-wide response to various WRRF configurations 148
6.3.3 Plant-wide Energy performance 151
6.3.4 GHG emission and carbon footprint 153

6.3.5 Economic evaluation.....155
6.3.6 General applicability of presented model.....156
6.4 DISCUSSION *160*
6.4.1 Ranking performance of anaerobic digestion configurations.....160
6.4.2 Importance of system boundary selection for energy performance analysis 163
6.4.3 VSS destruction and sludge disposal: drivers of GHG emissions and operational cost benefits.....163
6.5 CONCLUSION *164*
6.6 ACKNOWLEDGEMENTS *165*
6.7 SUPPORTING INFORMATION *166*
6.8 REFERENCES.....*183*

CHAPTER 7 ERROR! BOOKMARK NOT DEFINED.

GENERAL DISCUSSION AND CONCLUSIONS **ERROR! BOOKMARK NOT DEFINED.**

7.1 MAIN OBSERVATIONS PRESENTED IN THIS THESIS ..... ***Error! Bookmark not defined.***
7.2 IMPLICATIONS FOR NORTH AMERICAN MARKETS ***Error! Bookmark not defined.***
7.3 SUSTAINABILITY ISSUES .....***Error! Bookmark not defined.***
7.4 IMPLICATIONS FOR COLD CLIMATES.....***Error! Bookmark not defined.***
7.5 REDUNDANCY IN DESIGN SYSTEMS.....***Error! Bookmark not defined.***
7.6 FINAL WORDS .....***Error! Bookmark not defined.***
7.7 REFERENCES.....***Error! Bookmark not defined.***

LIST OF TABLES

Table 3.1 Operational conditions for the anaerobic digesters 41

Table 3.2. Components of energy flow model in anaerobic digesters 44

Table 3.3. Average performance of anaerobic digesters at steady-state during Phase I (Day 80-180) and Phase II (Day 250-350). 51

Table S3.1. Average characteristics of raw and ozonated waste activated sludge (WAS) 56

Table S3.2. Paired-observation randomized complete block design (RCBD) ANOVA comparing average values of VSS, soluble COD and biogas production under the different treatments for the two experimental phases. 58

Table 4.1. Methane yield in bottle reactors and BMP assays 72

Table 4.2. Methane yield in bottle reactors and BMP assays 73

Table S4.1. Average characteristics of raw and ozonated waste activated sludge (WAS) and anaerobic digestate (AD). 81

Table S4.2. Primer sequences and PCR thermal cycles of PCR 1 and PCR2. 82

Table S4.3. Richness and diversity estimators of bacterial communities in semi-continuously fed anaerobic digestion systems ($\alpha = 0.03$). 84

Table S4.4 Richness and diversity estimators of archaeal communities in semi-continuously fed anaerobic digestion systems ($\alpha = 0.03$). 84

Table S5.1. Primer sequences and PCR thermal cycles of PCR 1 and PCR2 114

Table S5.2 ANOVA p values testing differences between periods at different SRTs (10, 15 and 20 days) of operation parameter values. 119

Table S5.3 Least significant difference for reactors operated at SRT of 10, 15 and 20 days. 119

Table S5.4 ANOVA results for reactors operated at SRT=15 days and SRT>15 days 120

Table S5.5. Total raw and high-quality amplicon sequencing reads 121

Table S5.6 Alpha diversity of bacterial populations in the four anaerobic digesters. 124

Table S5.7 Alpha diversity for archaeal populations in the four anaerobic digesters. 125

Table 6.1. Description of the 6 modeling scenarios analyzed for RAEBL full-scale treatment facility with different operational AD configurations. 141

Table. 6.2. Comparison of digester performance, energy analysis and carbon footprint of typical full scale WRRF (default influent COD fractionations, kinetic and stoichiometric values from BioWin) for anaerobic digester at 35 °C fed with raw WAS and anaerobic digester at 20 °C fed with ozonated WAS. 156

Table 6.3. Ranking performance of the six configurations for four different indicators 160

Table S6.1. Influent fractions and model parameters used for simulation study. 164

Table S6.2. Energy consumption of different equipment's in RAEBL WRRF 166

Table S6.3. Components of energy flow model in anaerobic digesters 167

Table S6.4. Amount of sludge transported to disposal sites and their respective distance (in km) from the RAEBL plant 168

Table S6.5. Sources of direct and indirect GHG emissions for different WRRF scenarios integrating ozone and thermal hydrolysis pretreatment of biosolids combined with anaerobic digestion 169

Table S6.6. Major Axis Regression (MAR) of measured and simulated data for one-year of operation of the RAEBL WRRF (before installation of anaerobic digesters). 172

Table S6.7. Major Axis Regression (MAR) of measured and simulated data for Figs S6.3 and S6.4 over a period of 150 days of the RAEBL WRRF (after installation of anaerobic digesters). 175

Table S6.8. Major Axis Regression (MAR) of measured and simulated data over a period of 80 days (25^{th} April-11^{th} July 2017) of the lab-scale anaerobic digesters (Fig S6.5). 177

Table. S6.9. Energy components of the treatment units for the RAEBL WRRF under six operational scenarios. 179

Table S6.10. Operating parameters used for simulation study of the generalized model. 180

LIST OF FIGURES

Fig 2.1. Proposed metabolic pathway for methane production from anaerobic digestion of complex organic matter. Step 1: disintegration and hydrolysis of complex organic matter, Step 2: acidogenesis from sugars and amino acids, Step 3: acetogenesis from alcohols, volatile fatty acids (VFAs) and long chain fatty acids (LCFAs), and Step 4: methane production through acetoclastic and hydrogenotrophic methanogenesis (modified from Batstone and Jensen (2011). 15

Fig.2.2. Solid retention time (SRT)-temperature profile for anaerobic digestion. Conventional anaerobic digesters are operated at 30-37 °C for absolute minimum SRT ranging between 3-4 days. By decreasing the temperature, the minimum SRT increases (Buhr & Andrews, 1977). 17

Fig. 2.3. Schematic diagram of a) anaerobic digestion with sludge pre-ozonation b) anaerobic digestion with sludge post-ozonation. 22

Fig.2.4. COD flow, energy requirement and GHG emission in a typical wastewater treatment system operating an anaerobic digester (adapted from (Mannina et al., 2016). Arrows in dashed lines - COD flow; arrows in orange- energy requirements; arrows in blue – CH_4 emission, N_2O emission and equivalent CO_2 (eCO_2) emissions. 27

Figure 3.1. Three anaerobic sequencing batch reactors (ASBRs) operated at low (20 °C) & high (35 °C) temperatures, and under non-ozonated (No O_3) & ozonated (O_3) conditions 40

Fig 3.2. Performance of laboratory-scale anaerobic digesters under different operational conditions (low versus high temperatures; ozonated versus non-ozonated feed) showing a) VSS reduction b) Biogas production c) Soluble COD d) Total volatile fatty acids (VFA) concentrations. Phase I= Reactors were operated for 181 days based on operational conditions described in Table 1; Phase II= Contents of the three (3) reactors were mixed on Day 182, aliquoted back to each reactor and re-operated for 168 days based on conditions described in Table 49

3.1 to show reproducibility of phase I. The legend in panel a) also applies for b), c) and d); The average initial volatile suspended solid concentration of WAS was aproximately10,500 mg/L.

Figure 3.3. Comparison of energy budgets for different operational regimes a) Anaerobic digestion operated at 35 °C fed with raw WAS b) Anaerobic digestion operated at 20 °C fed with ozonated WAS (temperature of air, earth, and incoming sludge assumed to be 10 °C). *Note:* All energy units are reported in terms of biogas equivalent (GJ, biogas/d); ESI= Energy sustainability index; O_3= Ozone; GJ= Gigajoule; d= Day; += Energy gain; − = Energy expenditure. 54

Figure S3.1. Solubilisation of waste activated sludge at different ozone doses; Each point on curve represents an average of 3 replicates (error bars indicate standard errors and VSS concentration was ~10,000 mg/L). 56

Figure S3.2. Concentrations of short-chain carboxylic acids in test reactor (20 °C/ O_3) over the experimental period. 57

Fig 4.1. Methane yield from ozonated and non-ozonated substrates for batch-fed BMP assay at 20 °C (a) and 35 °C (b). Actual measured data are shown as marker dots and prediction by the modified Gompertz model is represented by the solid line. For the BMP assays, the samples were analyzed in quadruplicate (n=4). The obtained data were not corrected based on the blank. 71

Fig. 4.2. Genus level taxonomic classification of a) bacterial and b) archaeal populations in digesters operated at 20 °C receiving ozonated WAS (20 °C/ WAS O_3) or non-ozonated WAS (20 °C/ No O_3), or operated at 35 °C receiving non-ozonated WAS (35 °C/ No O_3). All digesters were operated at a 20-day SRT. Each phylum is coded with a unique color, and the height of each bar represents their abundance of reads. Phylogenetic groups accounting for ≤2% of all classified sequences are summarized in the artificial group “others”. 76

Fig. 4.3. Canonical correspondence analysis (CCA) showing correlations between a) Bacterial and b) Archaeal communities with environmental variables (VSS, 78

temperature, ozone, pH, soluble COD and VFA concentration). The process variables are represented by black arrows and the bottle digesters by different colored markers. Key bacterial and archaeal genera are shown as open circles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. S4.1. Rarefaction curves of bacterial and archaeal OTUs defined by 3% sequence variation in anaerobic digestate samples for 20 °C/ WAS O_3, 20 °C/ No O_3 and 35 °C/ No O_3 collected on different days. *Note:* 20 ºC-O_3 = digester fed with ozonated WAS and operated at 20 ºC, 35 ºC/NoO3 = digester fed with raw WAS and operated at 35 ºC, and 20 ºC-NoO_3 = digester fed with raw WAS and operated at 20 ºC; OTU = Operational taxonomic unit; D = denotes the sampling day. 83

Fig. S4.2 Phylum level taxonomic classification of a) bacterial and b) archaeal populations in digesters operated at 20 °C receiving ozonated WAS (20 °C/ WAS O_3) or non-ozonated WAS (20 °C/ No O_3), or operated at 35 °C receiving non-ozonated WAS (35 °C/ No O_3). All digesters were operated at a 20-day SRT. Each phylum is coded with a unique color, and the height of each bar represents their abundance of reads. Phylogenetic groups accounting for ≤2% of all classified sequences are summarized in the artificial group "others". 85

Fig. S4.3. Principal coordinate analysis (PCoA) of a) Bacterial and b) Archaeal communities of the bottle digesters. Ordination of samples was based on the Bray-Curtis distance metric at 3% cutoff level. Markers of the same type denote samples from the same reactors but at different sampling days. The circles show possible clustering of samples based on similar phylogenetic composition. 86

Fig. 5.1. Schematic overview of four anaerobic sequencing batch reactors (ASBRs) operated at a) 20 °C and fed with ozonated WAS and raw AD, b) 20 °C and fed with ozonated AD and raw WAS, c) 35 °C and fed with ozonated WAS and raw AD, and d) 35 °C fed with raw WAS (Control). Total reactor volume= 4 L, effective working volume=2 L. WAS-Waste activated sludge, AD-Anaerobic 96

digestate, O_3-ozonated substrate. The following identify the different components of the digesters: (1) pH probe; (2) Mechanical mixer; (3) temperature probe; (4) feeding/wasting line; (5) recirculation line.

Figure. 5.2. a) Volatile solids destruction, b) biogas production rate and c) Volatile fatty acid (VFA) and non VFA COD from anaerobic digestion of WAS and AD at different SRTs. WAS-waste activated sludge; AD-anaerobic digestate; O_3-ozonated substrate. Legend of panel a) also applies to panel b) and c). Error bars indicate standard errors among 3 time-intervals of 21 days for SRT 15d and 20d and 3 time-intervals of 14 days for SRT 10d, independent averages obtained after near-steady-state was reached. The significance of differences between the SRTs is reported in Table S5.2 and S5.3. 101

Fig. 5.3. a) Volatile solids destruction, b) biogas production rate, c) Volatile fatty acid (VFA) and d) non-VFA COD from anaerobic digestion of WAS and AD at SRT=15d and SRT>15d. WAS-waste activated sludge; AD-anaerobic digestate; O_3-ozonated substrate. Legend of panel a) also applies to panel b), c) and d). Error bars indicate standard errors among 3 time-intervals of 21 days for SRT 15d and >15d, independent averages obtained after near-steady-state was reached. The significance of differences between the SRTs is reported in Table S5.4. *Note*: For SRT>15d, the level of solids in the three ozonated digesters was maintained at the same level as in the 35 °C/ No O_3 digester. The SRT for the 35 °C/ WAS O_3, 35 °C/ No O_3, 20 °C/ WAS O_3 and 20 °C/ AD O_3 reactors in Period 5 was 24, 15, 19 and 21 days, respectively. 104

Fig. 5.4. Principal coordinate analysis (PCoA) of bacterial (a) and archaeal (b) *16S rRNA* gene amplicon Illumina sequencing data for anaerobic digestate samples at SRT of 15 days. Ordination was performed based on Bray-Curtis distance and OTUs were defined at 3% similarity. 106

Fig. 5.5. Distribution of eight (8) most abundant *Bacterial* and *Archaeal* genera in the four ASBRs operated at different SRTs. The genera belonging to the same phylum are grouped together. The height of each bar represents their relative 108

abundance. Digester a & e) 20 °C/ WAS O_3 b & f) 20 °C/ AD O_3, c & g) 35 °C/ WAS O_3 and d & h) 35 °C/ No O_3. Phylogenetic groups accounting for ≥2% of all classified sequences were considered. Each bar represents averages of the following days: SRT = 15 days: Day 105, 140 and 175, SRT = 10 days: Day 210 and 245, and SRT =20 days: Day 281 and 316. The *p* value presented above each group represents ANOVA between SRTs (α=0.05).

Fig 5.6. Effect of SRT-HRT decoupling on the distribution of eight (8) most abundant *Bacterial* and Archaeal genera in the four ASBRs operated for SRT=15 days and SRT>15 days. The genera belonging to the same phylum are grouped together. The height of each bar represents their relative abundance. Digesters a) 20 °C/ WAS O_3 b) 20 °C/ AD O_3, c) 35 °C/ WAS O_3 and d) 35 °C/ No O_3. Phylogenetic groups accounting for ≥2% of all classified sequences were considered. Each bar represents average of the following days: SRT = 15 days: Day 105, 140 and 175 (Period 3), and SRT >15 days (Period 5): Day 350 and 385. The *p* value presented above each group represents the ANOVA between SRTs (α=0.05). 112

Figure S5.1. Solubilization of waste activated sludge (WAS) and anaerobic digestate (AD) at different ozone doses. Each point on the curve represents an average of 3 replicates (error bars indicate standard errors; n=3). The samples used for the solubilization experiment contained a VSS concentration of ~30,000 and 20,000 mg/L for WAS and AD, respectively. For panel a and c the Y axis shows the initial soluble COD at ozone dose of 0 and panels b and d the Y axis is the increase in sCOD concentration (sCOD at ozone dose (t) - sCOD at ozone dose (0)). 116

Fig. S5.2. VSS concentration (a), biogas production rate (b), Soluble COD concentration (c) and NH_4^+-N concentration (d) during anaerobic digestion of WAS and digestate at different SRTs. Period 1: start-up period- SRT=20 days, Period 2: Decreasing SRT from 20 to 15 Period 3: decreasing SRT to 10 days, Period 4: SRT=20 days, Period 5: Decoupling of SRT and HRT in the ozonated 117

reactors. The SRT for the 35 °C/ WAS O_3, 35 °C/ No O_3, 20 °C/ WAS O_3 and 20 °C/ AD O_3 reactors in Period 5 was 24, 15, 19 and 21 days, respectively. Average values were calculated among 3 time-intervals of 21 days for SRT 15d and 20d and 3 time-intervals of 14 days for SRT 10d, independent averages obtained after near-steady-state was reached.

Figure S5.3. Level of volatile fatty acids (VFAs) in the anaerobic digesters at different SRTs. Each point on the graph represents an average of 3 replicates. Error bars indicate standard error (n=3). Period 1: start-up period- SRT=20 days, Period 2: Decreasing SRT from 20 to 15 Period 3: decreasing SRT to 10 days, Period 4: SRT=20 days, Period 5: Decoupling of SRT and HRT in the ozonated reactors. The SRT for the 35 °C/ WAS O_3, 35 °C/ No O_3, 20 °C/ WAS O_3 and 20 °C/ AD O_3 reactors in Period 5 was 24, 15, 19 and 21 days, respectively. 118

Fig. S5.4. Rarefaction curves of bacterial OTUs defined by 3% sequence variation in anaerobic digestate samples for (a) 20 °C/ WAS O_3 (b) 20 °C/ AD O_3 (c) 35 °C/ WAS O_3 (d) 35 °C/ No O_3. Legend in panel a) applies to the other panels as well. 122

Fig. S5.5. Rarefaction curves of archaeal OTUs defined by 3% sequence variations in anaerobic digestate samples for (a) 20 °C/ WAS O_3 (b) 20 °C/ AD O_3 (c) 35 °C/ WAS O_3 (d) 35 °C/ No O_3. Legend in panel a) applies to the other panels. 123

Figure S5.6. Relative abundance of phylogenetic groups at phylum level in the anaerobic digesters operated at different conditions (20 °C vs 35 °C; SRT of 10, 15 and 20 days; and ozonated vs non-ozonated feed) for bacteria (a) and archaea (b). Each phylum is coded with a unique pattern, and the height of each bar represents their respective relative abundance. Phylogenetic groups accounting for ≤2% of all classified sequences are summarized in the artificial group “others”. In panel (b) the break range for the Y axis is 20-90% with 5% intervals after the break. Each bar represents averages of the following days: SRT = 15 days: Day 105, 140 and 175, SRT = 10 days: Day 210 and 245, and SRT =20 days: Day 281 and 316. 126

Fig.S5.7. Relative abundance of major phylogenetic groups at phylum level detected in the anaerobic digesters under coupled or decoupled SRT-HRT conditions (Period 3-SRT=15 days, Period 5-SRT>15 days). Bacterial phyla are presented in panel (a) and the archaeal phyla are presented in panel (b). Phylogenetic groups accounting for ≤2% of all classified sequences are summarized in the artificial group "Others". In panel (b) the break range for the Y axis is 20-90% with 5% intervals after the break. Note: The SRT for the 35 °C/ WAS O_3, 35 °C/ No O_3, 20 °C/ WAS O_3 and 20 °C/ AD O_3 reactors in Period 5 was 24, 15, 19 and 21 days, respectively. Each bar represents average of the following days: SRT = 15 days: Day 105, 140 and 175, and SRT >15 days (Period 5): Day 350 and 385. 127

Fig 6.1. Schematic of the RAEBL WRRF with the main wastewater treatment stream (top) and the anaerobic digestion and sludge handling stream (bottom) and potential GHG Emission at different treatment stages. In the wastewater stream, the bioreactors HRT and SRT were 15hr, and 7 days, respectively. In the sludge stream, the hydrolyzer was operated at 55 ºC with an SRT of 48 h, and the digester was maintained at 35-37 ºC with an SRT of 19 days. Detailed descriptions are presented in the text. 138

Fig 6.2. Scenario analysis of anaerobic digesters at different temperatures (20 °C and 35 °C) and pretreatments (thermal hydrolysis [HD] and WAS ozonation [O_3]). A) VSS destruction (%); b) Biogas flowrate (%); c) Anaerobic digestate NH_4^+-N (%), The bar charts indicate the percentage of (Average Simulated /Average Measured)-1 [%]. The base case scenario involved digester operation at 35 °C with thermophilic hydrolysis at 55 °C (35 °C/ WAS HD); Period of plant data: July-Dec 2017; average values of measured data for the base case scenario: VSS destruction: 41±2%; Biogas flowrate: 105±1 m^3/h; NH_4^+: 1423±16 mg-N/L; The error bars indicate the standard error measured at steady state. 148

Fig 6.3. Energy budget (expenditure and gain) for different operational scenarios (20 °C and 35, and with or without pretreatment). Stacked bars showing positive 150

energy refer to energy gain while bars displaying negative energy relate to energy consumed by the system. The net energy of the operational configurations is shown as a solid line superimposed on the stacked bar chart. Note: Energy for cooling AD represents the energy required for cooling down the temperature of the sludge leaving the thermophilic hydrolyzer (55 °C) and entering the AD (35 °C).

Fig 6.4. Total direct and indirect emissions for different operational scenarios of anaerobic digesters (20 °C and 35 °C, and with or without thermal hydrolysis and ozone pretreatment). The solid line depicts the total carbon footprint of the analyzed scenarios. 152

Fig 6.5. Operational cost analysis for different operational scenarios (20 °C and 35 °C, and with/without thermal hydrolysis and ozonation pretreatment). Positive values indicate income while negative values represent expenditures. 154

Fig. S6.1. Simulation of a) MLVSS b) effluent NH_4^+-N and c) effluent NO_3^- concentration of RAEBL WRRF. Major axis regression (MAR) of measured and predicted values for d) MLVSS e) effluent NH_4^+-N and f) effluent NO_3^- concentration. Simulation was performed using one year of operational data from Jan 1st, 2016 (Day 1) to Dec 31st, 2016 (Day 365) before the installation of anaerobic digesters. 171

Fig. S6.2. Simulation of a) MLVSS b) effluent NH_4^+-N and c) effluent NO_3^--N concentration of RAEBL WRRF. Major axis regression (MAR) of measured and predicted values for d) MLVSS e) residual NH_4^+-N and f) residual NO_3^-. Simulation was performed using 6 months of operational data from July 22nd 2017 (Day 1) to Dec 31st 2017 (Day 150) after installation of anaerobic digesters. 173

Fig. S6.3. Simulation of a) anaerobic digestate VSS b) biogas flowrate and c) digestate NH_4^+-N concentration of RAEBL WRRF. Major axis regression (MAR) of measured and predicted values for d) anaerobic digestate VSS e) biogas flowrate and f) residual digestate NH_4^+-N. Simulation was performed using 6 months of 174

operational data from July 22nd 2017 (Day 1) to Dec 31st 2017 (Day 150) after installation of anaerobic digesters.

Fig. S6.4. Simulation of a) Anaerobic digestate VSS b) Biogas flowrate c) Digestate NH_4^+-N concentration for ASBRs (2000 mL, Chapter 5) operated at 20 °C and 35 °C. The solid lines and makers show the model simulations and measured data, respectively. For the scenario of AD at 20 °C with raw WAS (20 °C/ No O_3), the simulated profile was conducted on 800 mL lab scale reactors described in Chapter 3. The concentration of WAS fed to the 2,000 mL and 800 mL lab scale digesters were 30,000 and 10,000 mg/L, respectively. No measured data was available for NH_4^+ concentration of 20 °C/ No O_3 (25th April-11th July 2017). 176

Fig. S6.5. Scenario analysis of anaerobic digesters at different temperatures (20 °C and 35 °C) and different pretreatment (thermal hydrolysis and ozonation) The solid lines show the model simulations and the red marker shows the measured data for the base case scenario (35 °C/ WAS HD). 178

Fig 7.1. Biogas production in North America vs. Europe (ABC, 2016; EBA, 2016). 190

LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations

ADM1	Anaerobic Digestion Model No. 1
ASBRs	Anaerobic Sequencing Batch Reactors
AD	Anaerobic Digestate
ASDM	Activated Sludge Anaerobic Digestion Models
ASMs	Activated Sludge Models
BMP	Biochemical Methane Potential
BTG	Biosolids Task Group
CCA	Canonical Correspondence Analysis
CBA	Canada Biogas Association
COD	Chemical Oxygen Demand
COD_{In}	Inlet Chemical Oxygen Demand
CCME	Canadian Council of Ministers of the Environment
DNA	Deoxyribonucleic acid
DO	Dissolved Oxygen
DAF	Dissolved Air-Flotation
eCO_2	Equivalent CO_2
EBMs	Energy Balance Models
EF	Emission Factor
EROI	Energy Return on Investment
ESI	Energy Sustainability Index
EPT	Energy Payback Time
EPS	Extracellular Polymeric Substances
EPA	Environmental Protection Agency
E_{CH4}	Energy from biogas
E_{req}	Energy required to heat up the digester
E_{mix}	Energy for mixing
E_{rec}	Heat recovery
E_{loss}	Heat loss

E_{net}	Net energy production
E_{O3}	Energy for ozonation
E_p	Total energy produced
E_c	Total energy consumed
GC-FID	Gas Chromatography with Flame Ionization Detector
GHG	Greenhouse Gases
GWP	Global Warming Potential
h_i	Heat Transfer Coefficients
H	Shannon's Diversity Index
HD	Thermal hydrolysis
HRT	Hydraulic Retention Time
INF	Influent
ISR	Inoculum-Substrate Ratio
IWA	International Water Association
IPCC	Intergovernmental Panel on Climate Change
J	Evenness
LCFA	Long chain fatty acid
LSD	Least Significant Difference
MANOVA	Multivariate Analysis of Variance
MAR	Major Axis Regression
MDDEP	Ministère du Développement Durable, de l'Environnement et des Parcs
ML	Mixed liquor
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
NC	Negative Control
OTU	Operational Taxonomic Unit
OUR	Oxygen Uptake Rate
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
RAS	Return Activated Sludge
RNA	Ribonucleic acid

RCBD	Randomized Complete Block Design
RMSE	Root Mean Square Error
S	Relative abundances of the OTUs
SMP	Specific Methane Production
SMP_{VSSin}	Specific Methane Production of initial VSS
$SMP_{VSSdestroyed}$	Specific Methane Production of VSS destroyed
STP	Standard temperature and pressure
sCOD	Soluble Chemical Oxygen Demand
$sCOD_{out}$	Soluble Chemical Oxygen Demand going out of the digester
SF	Safety Factor
SRT	Solids Retention Time
$[SRT_{min}]_{lim}$	Absolute Minimum Solids Retention Time
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TP	Total Phosphorus
T_{air}	Ambient temperature
T_{dig}	Digester temperature
T_{ground}	Temperature of ground
T_{in}	temperature of incoming sludge
$T_{exc\text{-}hot}$	Hot temperature coming out of the heat exchanger
$T_{exc\text{-}cold}$	Cold temperature going in the heat exchanger
TS	Total Solids
TSS	Total Suspended Solids
VFA	Volatile Fatty Acids
VS	Volatile Solids
VS_{in}	Input Volatile Solids
VSS	Volatile Suspended Solids
VSS_{added}	Added Volatile Suspended Solids
VSS_{in}	Input Volatile Suspended Solids
WAS	Waste Activated Sludge
WRRF	Water Resource Recovery Facility

WWTP	Wastewater Treatment Plant

Symbols	Modeling parameters	Units
f_{SB}	Soluble biodegradable organics	g-COD_{SB} / g-Total COD
f_{XU}	Particulate undegradable organics	g-COD_{XU} / g-Total COD
f_{SU}	Soluble undegradable organics	g-COD_{SU} / g-Total COD
f_{XCB}	Slowly biodegradable substrates	g-COD_{XCB} / g-Total COD
f_{XOHO}	Ordinary heterotrophic biomass	g-COD_{XOHO}.m^{-3}
f_{XANO}	Nitrifying biomass	g-COD_{XANO} / g-Total COD
$f_{SU\_O3,trans}$	Soluble undegradable COD ()	g-COD_{SU}.g-COD_{XS}^{-1}
$f_{SB\_O3,trans}$	Soluble biodegradable COD ()	g-COD_{SB}.g-COD_{XS}^{-1}
$f_{XCB,O3,trans}$	Particulate biodegradable COD ()	g-COD_{XCB}.g-COD_{XS}^{-1}
$f_{mnr,O3}$	Oxidized COD ()	g-$COD_{mnr.g}$-COD_{XS}^{-1}
XC_B	Slowly biodegradable substrates	g-COD_{XCB}/m^3
X_{ND}	Slowly biodegradable organic nitrogen	g-COD_{XND}/m^3
SRT_{min}	Minimum solids retention time	days
TKN_{Inf}	Influent Total Kjeldahl Nitrogen	mg-N/L
T	Temperature	°C
μ_{max}	Max. specific growth rate	d^{-1}
b	Decay rate	d^{-1}
q_{am}	Ammonification rate	g-COD/m^3/d
q_{ozone}	Specific rate of ozonation	d^{-1}
θ	Temperature coefficient	--
θ_h	Hydraulic retention time	days
θ_x	Solids retention time	days
$\theta_{x,design}$	Design solids retention time	days
$[\theta_{x,min}]$	Minimum solids retention time	days
$[\theta_{x,min}]_{lim}$	Absolute minimum solids retention times	days
$\theta_{x\ min,\text{with ozone}}$	Minimum solids retention time with ozonation	days
V_{dig}	Volume of the digester	m^3

ρ_{sludge}	Density of sludge	kg/m^3
c_{sludge}	Specific heat of sludge	J/kg °C
η_{el}	Electrical efficiency	--
Y_{CH4}	Cumulative methane yield	mL CH_4/g VS_{in}
$Q_{\text{contactor}}$	biosolids flow rate in the ozone contactor	m^3/d
$X_{\text{contactor}}$	Biosolids concentration in the ozone contactor	mg/L
X_{dig}	Biosolids concentration in the digester	mg/L
P	Ultimate methane yield	mL CH_4/g VS_{in}
r_m	Maximum methane production rate	mL CH_4/g VS_{in}.d
λ	Lag period	days
t	Digestion time	days

ABSTRACT

The production and disposal of large volumes of biosolids by municipal wastewater treatment infrastructures represent an important risk to the environment and an economic burden to plant operators considering that annual cost of biosolids management is approximately 50% of the total operation cost of wastewater treatment facilities. Anaerobic digestion is one of the most widely used processes to treat biosolids prior to their disposal or re-use for land application, and has the added benefit of producing valuable biogas and energy. Conventionally, anaerobic digesters are operated at 35 ºC to overcome the rate-limiting step of hydrolysis converting complex polymers into simpler molecules for uptake by the digesting microbial biomass. However, the energy expenditure for heating up the anaerobic digester system is significant. This study assessed the feasibility of operating anaerobic digesters at low mesophilic temperature (20 ºC) by combining them with sludge ozonation. An initial investigation consisted of operating three anaerobic reactors for 350 days. The findings showed that performing solids ozonation prior to anaerobic digestion at 20 ºC led to a higher volatile suspended solids (VSS) destruction of 35% than conventional anaerobic digestion at 35 ºC with raw sludge. The methane production was also enhanced from 200.5 mL CH_4/g VSS_{in} to 232.1 mL CH_4/g VSS_{in} for the 35 ºC digester without sludge ozonation. Energy balance calculations showed that the 20 ºC-ozonated digester produced 35% more energy than the 35 ºC digester, with a net energy balance of +174 GJ/d and +129 GJ/d, respectively, thereby suggesting a more energetically sustainable option for treatment of municipal biosolids.

Biochemical methane potential (BMPs) assays were used to determine the extent of solids degradation induced by ozonated feedstocks and compare the kinetics of methane yield and maximum methane production rate between anaerobic digestion under conventional conditions (35 °C) and low mesophilic temperature (20 °C) using ozonated waste activated sludge (WAS) and anaerobic digestate (AD). A higher methane yield was obtained at both temperatures for the batch system using ozonated substrates compared to non-ozonated feed. rRNA gene amplicon sequencing using high-throughput next generation DNA sequencing revealed distinct bacterial and archaeal community structures and composition between digesters fed with ozonated and non-ozonated substrates at both temperature regimes. Temperature and feed type were found to play an important role in shaping the microbial diversity and community structure, which were closely linked to the functional stability and performance of the digesters.

Further insights were gained by operating four lab-scale anaerobic sequencing batch reactors (ASBRs) to study the effect of sludge pretreatment by ozonation and low mesophilic temperature (20 ºC) on anaerobic digestion. The hybrid system combining sludge ozonation at low mesophilic temperature (20 ºC) was found to display a better digester performance with an enhanced volatile suspended solids (VSS) reduction by 20% and biogas production by 29% as compared to conventional anaerobic digestion at 35 °C with untreated sludge. Ozonating the anaerobic digestate (AD) rather than the waste activated sludge (WAS) increased the VSS reduction and biogas production by almost 10% showing that the point of ozonation is also an important factor to consider when implementing low temperature anaerobic digestion. Variation in solids retention time (SRT) clearly affected the reactor performance due to accumulation of volatile fatty acids (VFAs) at low SRTs. Decoupling the SRT from the hydraulic retention time (HRT) significantly improved the VSS reduction and methane yield at low temperature. Microbial community analyses showed discernible differences in bacterial and archaeal populations between the studied anaerobic digesters. Digesters operated at low temperature (20 °C) and fed with ozonated substrates displayed a high dominance of *Clostridium*, while the digester at 35 °C showed a higher abundance of *Ruminococcus*. Shortening of SRT was found to induce the hydrogenotrophic pathway while decoupling the SRT from the HRT favoured the acetoclastic pathway for methane production. These key elements are important for parameterizing and optimizing anaerobic digestion at low mesophilic temperature (20 °C) combining sludge ozonation to derive maximum benefits from the system in terms of VSS reduction and biogas production.

Plant-wide modeling showed that integrating sludge ozonation imparted added benefits to anaerobic digestion including feasibility at low mesophilic temperature (20 ºC), enhanced digester performance in terms of VSS reduction and biogas production, higher energetic sustainability and reduced carbon footprint and operational cost, than conventional anaerobic digestion at 35 ºC. The assessment of a full-scale treatment facility provided evidence that the proposed new configuration combining sludge ozonation at low mesophilic temperature presents higher energy efficiency as well as environmental benefits in terms of lower direct and indirect greenhouse gas (GHG) emissions and reduced economic impacts. The future development of this proposed technology would require refining the sustainability assessment to better substantiate arguments for its full-scale commercialization.

RÉSUMÉ

La production et l'élimination de grandes quantités de biosolides par les infrastructures municipales de traitement des eaux usées représentent un risque important pour l'environnement et un fardeau économique pour les exploitants, étant donné que le coût annuel de la gestion des biosolides représente environ 50% du coût total d'exploitation des installations de traitement des eaux usées. La digestion anaérobie est l'un des procédés les plus utilisés pour traiter les biosolides avant leur élimination ou réutilisation à des fins agricoles. Elle offre l'avantage supplémentaire de produire du biogaz et de l'énergie. D'habitude, les digesteurs anaérobies fonctionnent à 35 °C afin de surmonter l'étape limitante de la conversion par hydrolyse des polymères complexes en molécules simples pour être absorbé par la biomasse microbienne. Cependant, l'énergie nécessaire pour chauffer le système est importante. La présente étude a évalué la faisabilité d'opérer des digesteurs anaérobies à basse température mésophile (20 °C) en les combinant avec une ozonation des boues. Une première enquête a consisté à exploiter trois réacteurs anaérobies pendant 350 jours. Les résultats ont démontré que l'ozonation des solides avant la digestion anaérobie à 20 °C entraînait une destruction plus importante des matières volatiles en suspension (MVS) de 35% par rapport à la digestion anaérobie conventionnelle à 35 °C avec des boues non-traitées. La production de méthane a également été augmentée de 200.5 mL de CH_4/g de MVS_{in} à 232.1 mL de CH_4/g de MVS_{in} pour le digesteur à 35 °C sans ozonation des boues. Les calculs du bilan énergétique ont montré que le digesteur ozoné à 20 °C produisait 35% plus d'énergie que le digesteur à 35 °C, avec un bilan énergétique net de +174 GJ/j et +129 GJ/j, suggérant ainsi une option plus durable sur le plan énergétique pour le traitement des biosolides municipaux.

Des analyses du potentiel biochimique en méthane (PBM) ont été utilisées afin de déterminer l'ampleur de la dégradation des solides induite par les matières premières ozonées et pour comparer la cinétique de rendement en méthane et le taux de production maximal de méthane entre la digestion anaérobie dans des conditions classiques (35 °C) et à basse température mésophile (20 °C) utilisant de boues activées résiduaires ozonées et de digestat anaérobie. Un rendement plus élevé en méthane a été obtenu dans les deux cas de températures pour le système utilisant des substrats ozonés par rapport aux boues non ozonées. Les analyses par séquençage d'amplicon de gènes d'ARN ribosomal à haut débit ont révélé une structure et composition de communautés bactériennes et archéales distinctes entre les digesteurs alimentés avec des substrats ozonés et non

ozonés pour les deux régimes de température. La température et le type de substrat ont donc joué un rôle important dans la formation de la diversité et structure des communautés microbiennes, qui étaient étroitement liés à la stabilité fonctionnelle et à la performance des digesteurs.

Des connaissances supplémentaires ont été obtenues en exploitant quatre réacteurs séquentiels anaérobies à l'échelle de laboratoire afin d'étudier les effets du prétraitement des boues par ozonation et de la basse température mésophile (20 °C) sur la digestion anaérobie. Le système hybride combinant l'ozonation des boues à basse température mésophile (20 °C) s'est avéré mieux performant pour le digesteur avec une réduction accrue de 20% des matières volatiles en suspension (MVS) et une production de biogaz de 29% par rapport à une digestion anaérobie classique à 35 °C avec des boues non-traitées. L'ozonation du digestat anaérobie plutôt que des boues activées résiduelles a augmenté de près de 10% la réduction du MVS et la production de biogaz, ce qui démontre que le point d'ozonation est également un facteur important à prendre en compte lors de l'élaboration d'un system de digestion anaérobie à basse température. La variation du temps de rétention des solides (TRS) a clairement affecté les performances du réacteur en raison de l'accumulation d'acides gras volatils (AGV) à faible TRS. Le découplage du TRS au temps de rétention hydraulique (TRH) a considérablement amélioré la réduction du MVS et le rendement en méthane à basse température. Les analyses de la communauté microbienne ont montré des différences perceptibles dans les populations bactériennes et archéales entre les digesteurs anaérobies étudiés. Les digesteurs fonctionnant à basse température (20 °C) et alimentés avec des substrats ozonés présentaient une forte dominance de *Clostridium*, tandis que le digesteur à 35 °C présentait une plus grande abondance de *Ruminococcus*. Il a été constaté que le raccourcissement du TRS induisait la voie hydrogénotrophe tandis que le découplage du TRS au TRH favorisait la voie acétoclastique pour la production de méthane. Ces éléments clés sont importants pour le paramétrage et l'optimisation de la digestion anaérobie à basse température mésophile (20 °C) combinant l'ozonation des boues afin de tirer le maximum d'avantage du système en termes de réduction des boues et de production de biogaz.

La modélisation à l'échelle de l'usine a démontré que l'intégration de l'ozonation des boues apportait des avantages supplémentaires à la digestion anaérobie, notamment la faisabilité à une température mésophile (20 ºC), l'amélioration des performances du digesteur en termes de réduction de MVS et de production de biogaz, de durabilité énergétique supérieure, de réduction

de l'empreinte carbone et de coût opérationnel comparé à la digestion anaérobie classique à 35 ºC. L'évaluation d'une installation de traitement à grande échelle a démontré que la nouvelle configuration proposée combinant l'ozonation des boues à basse température mésophile présente une efficacité énergétique accrue ainsi que des avantages environnementaux en termes de réduction des émissions de gaz à effet de serre (GES) directes et indirectes ainsi que des impacts économiques réduits. Le développement futur de la technologie proposée nécessiterait un affinement sur l'évaluation de la durabilité afin de mieux justifier les arguments en faveur de sa commercialisation à grande échelle.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my supervisor, Prof. Dominic Frigon, for his guidance and support. I am also grateful to Dr. Jean-Francois Lemay, Dr. Antonio Avalos Ramirez and Patrik Quessy from the Centre National en Électrochimie et en Technologie Environnementales Inc. (CNETE) for their valuable assistance in performing part of the physiochemical analyses and the biomethane potential (BMP) assays. I would like to thank my colleagues of the Microbial Community Engineering Laboratory group, especially Shameem Jauffur for his everlasting assistance, friendship and support. My heartfelt thanks to Daina Brumelis, Manager of the Environmental Engineering Laboratory, for her constant technical advice. I also wish to extend my thanks to the administrative staff of the Department of Civil Engineering and Applied Mechanics. I am also indebted to my family for their constant support and endless love. Finally, I would like to deeply acknowledge the following funding agencies for supporting my research and without which this study would not have been possible: McGill Engineering Doctoral Award (MEDA), McGill Graduate Excellence Award, Natural Sciences and Engineering Research Council of Canada (NSERC), Mitacs Internship programs, Air Liquide Canada and the Régie d'Assainissement des Eaux du Bassin LaPrairie (RAEBL).

PREFACE

In accordance with the "*Guidelines for Thesis Preparation*", this thesis is presented in a manuscript-based format. A general introduction and literature review are presented in Chapters 1 and 2. Chapters 3-6 comprise of one published article, and three research articles which are in preparation and will be submitted for publication to the journals of *Bioresource Technology*, *Environmental Science & Technology* and *Bioresource Technology*, respectively. The author of this thesis is the primary author of all the manuscripts. In the final Chapter, a General Discussion and Conclusions are presented. Below is a detailed description of the efforts of each contributing author.

Zeinab Bakhshi, Shameem Jauffur and Dominic Frigon, Assessing energy benefits of operating anaerobic digesters at low temperature with solids pre-ozonation. *Journal of Renewable Energy*, 2018, Volume 115, Pages 1303-1311.

Authors' contributions:

Zeinab Bakhshi: Designed the study, conducted the experimental procedures, analyzed the results, and wrote the manuscript.

Shameem Jauffur: Helped with the study design, assisted with the collection of samples and revised the manuscript.

Dominic Frigon: Supervised the research, helped with the study design, and revised the manuscript.

Zeinab Bakhshi, Shameem Jauffur, Patrik Quessy, Jean-François Lemay and Dominic Frigon "Effect of sludge ozonation on kinetics and biogas recovery in batch-fed and semi-continuous anaerobic digestion systems at low mesophilic temperature". To be submitted to the journal of *Bioresource Technology* (In preparation).

Authors' contributions:

Zeinab Bakhshi: Designed the study, conducted the experimental procedures, analyzed the results, performed the modeling, and wrote the manuscript.

Shameem Jauffur: Assisted with the collection of samples from water resource recovery facilities (WRRFs), helped with the design and operation of the anaerobic digesters and revised the manuscript.

Dominic Frigon: Supervised the research, helped with the study design, and revised the manuscript.

Jean-François Lemay: Conducted part of the BMP tests, helped with the study design, and revised the manuscript.

Patrik Quessy: Conducted part of the BMP tests.

Zeinab Bakhshi, Shameem Jauffur and Dominic Frigon. "Maximization of energy recovery and reduction of biosolids production by combining ozonation treatment and anaerobic digestion at low mesophilic temperature". To submit to the journal of *Environmental Science & Technology* (In preparation).

Authors' contributions:

Zeinab Bakhshi: Designed the study, conducted the experimental procedures, analysed the results, and wrote the manuscript.

Shameem Jauffur: Assisted with the collection of samples and helped with the molecular DNA and bioinformatic analyses.

Dominic Frigon: Supervised the research, helped with the study design, and revised the manuscript.

Zeinab Bakhshi, Shameem Jauffur and Dominic Frigon. "Plant-wide modeling of anaerobic digestion combining sludge ozonation at low mesophilic temperature: Exploring energy, carbon footprint and cost benefits". To be submitted to the journal of *Bioresource Technology* (In preparation).

Authors' contributions:

Zeinab Bakhshi: Designed the study, conducted the experimental procedures, operated the reactors, constructed the plant-wide models, performed the energy balance and carbon footprint analyses, analysed the results and wrote the manuscript.

Shameem Jauffur: Assisted with the model construction and carbon footprint analyses, and revised the manuscript.

Dominic Frigon: Supervised the research, helped with the study design and revised the manuscript.

CHAPTER 1

Introduction

1.1 INTRODUCTION

Municipal biosolids and waste sludge represent residual materials generated from the biological processing of wastewater in water resource recovery facilities (WRRFs). According to the *Guidelines for Effluent Quality and Wastewater Treatment at Federal Establishments*, the disposal of untreated biosolids to the environment is unacceptable, and appropriate disposal practices should be adopted to ensure the protection of the receiving environment and public health (CCME, 2010a). Disposal of biosolids involves significant costs, which can account for up to 50-60% of the total operational budget of biological WRRFs (Liu, 2003). This cost can be further accrued if the biosolids are converted to 'Class A' category to meet the U.S Environmental Protection Agency (EPA) guidelines or the Canadian Council of Ministers of the Environment (CCME) Framework for wastewater biosolids for land application with no restrictions, and involves stabilization and disinfection prior to use as fertilizers or compost (Anjum et al., 2016).

In Quebec, the imposition of the landfill disposal tax has triggered a dramatic increase in the disposal costs of sewage biosolids from $30/ton to over $100/ton in 2008 (LeBlanc et al., 2009). Furthermore, according to the 2011 provincial regulation, landfilling and incineration of municipal biosolids (representing >4 million metric tons per year of putrescible organic waste in Quebec) will be completely banned by the year 2022 as part of the Government plan to reduce the emission of greenhouse gases (GHGs) from the waste sector (Villeneuve & Dessureault, 2011). Consequently, wastewater utilities are turning more and more towards implementation of biosolids minimization technologies such as composting, spreading of biosolids as fertilizing residual materials and biogas generation. According to the Biosolids Task Group (BTG) of the Canadian Council of Ministers of the Environment (CCME), state-of-the-art research should be conducted on wastewater residuals to promote new and robust technologies in view of bringing new changes in biosolids treatment and disposal practices that can enable opportunities for beneficial and sustainable use options (CCME, 2012). The work presented in the current thesis is an answer to this call for the development of new technologies.

Anaerobic digestion of waste activated sludge (WAS) is one of the most common processes used for biosolids stabilization and reduction. The process typically converts about 50% of the WAS organic matter into methane gas. This allows the recovery of energy and improves the sustainability of WRRFs (Bougrier et al., 2007). The anaerobic transformation of organic

particulates comprises four general sequential steps: (1) disintegration and hydrolysis producing simple organic monomers, (2) acidogenesis fermenting monomers into hydrogen and short-chain volatile fatty acids (VFAs), (3) syntrophic acetogenesis completing the transformation of VFAs into acetate, and (4) hydrogenotrophic and acetoclastic methanogenesis, utilizing hydrogen and acetate to produce methane (Batstone & Jensen, 2011). Anaerobic digestion of biosolids is slow and the hydrolysis of particulate organic matter is considered as the rate-limiting step in the degradation process (Mottet et al., 2013). This is why high-rate mesophilic anaerobic digestion systems are typically operated at a minimum of 35 °C to increase the hydrolysis rate of slowly degradable organic matter (Rittmann & McCarty, 2001c).

Several physicochemical pretreatment methods have been developed and implemented to increase the hydrolysis rate, such as ultrasound disintegration (Tiehm et al., 2001), alkaline hydrolysis (López Torres & Espinosa Lloréns, 2008), thermal disintegration at high pressure (600- 2500kPa) (Bougrier et al., 2008) and oxidative hydrolysis (Bougrier et al., 2007). All these pretreatments aim at disintegrating the biosolids, solubilizing organic matter, and transforming non-degradable components into degradable ones for easy uptake by microorganisms (Carrere et al., 2010). Such strategy is useful as it reduces the solid retention times (SRTs) or the digester volume thereby increasing volumetric methane productivity and lowering capital investments (Tiehm et al., 2001). Although these pretreatment techniques consume high levels of energy for cell disintegration, they decrease final sludge handling and disposal costs especially for disposal options such as landfilling and incineration, and enhance energy recovery in the form of biogas. Based on a report by the CBA (2013), almost 180 Mm^3/year of biogas are generated from anaerobic digesters in Canada and used to produce 60 MW of green electricity. Still, the CBA recommends that the potential of anaerobic digestion be further explored to increase the recovery of biogas from wastewater treatment facilities, while reducing the amount of digestate to dispose of and the energy footprint of digesters.

Ozonation is one of the techniques that has been used to improve the hydrolysis of biosolids for anaerobic digestion. Ozone is a powerful oxidant for a wide range of organic and inorganic compounds (Chu et al., 2008). WAS ozonation has been shown to transform refractory organics into biodegradable compounds and substantially decrease the volume of disposed biosolids and produce more methane (Goel et al., 2003a). Ozone reacts with sludge flocs and transforms the

associated COD into different pools. It exerts a lytic effect on microbial cells, releasing intracellular compounds, which are utilized as substrates by the microbial biomass. The particles are solubilized, biomass is inactivated and non-biodegradable particulate organics are transformed into biodegradable substrates, relieving the rate-limiting step of hydrolysis (Isazadeh et al., 2014).

From an operational point of view, it is important to assess the energy footprint of anaerobic digesters to optimize their energy recovery performance (Chynoweth et al., 2001). The highest energy consumption component in the anaerobic digestion process is the heating of the digester for optimum metabolism of the digesting microbial community (Navickas et al., 2013). In Canada, operating anaerobic digesters at the high mesophilic temperature range (30-37 °C) implies a significant energy expenditure because the influent WAS from aerobic treatment is at a much lower temperature especially during winter (5-15 °C). According to Grant and Lin (1995), depending on the temperature of the matrix to be treated and the particular climatic conditions, it is not always practical to operate digesters at the optimum temperature because of the high energy requirements. Puchajda and Oleszkiewicz (2008) estimated that the operation of an anaerobic digester at 35 °C would require an energy expenditure of about 47% of the biogas produced, in order to heat the reactor. Theoretically, operating anaerobic digesters at lower temperature would involve a lower energy expenditure. However, in practice, such low-temperature operation is not feasible due to the low rate of organic matter hydrolysis and biogas production. The technology of anaerobic digestion of organic solid wastes has, no doubt, matured over the past years in many aspects including fundamentals (kinetics and modeling), process performance (single and dual stage systems, wet and dry technologies), digestion enhancement (pretreatments), co-digestion with other substrates, and its relation to solids composting. Now, the main challenge has turned towards decreasing digestate production, reducing its energy footprint, limiting emission of GHGs and maximizing energy recovery through biogas production.

At the inception of the work presented in this thesis, it was hypothesized that ozonation can enhance the performance of anaerobic digestion of WAS by increasing the degradability of biosolids and increasing the hydrolysis and disintegration rate when the operational temperature is reduced from 35 °C to 20 °C (or lower). If such an approach is feasible, it will not only provide a technological solution to reduce excess sludge production but may produce an equivalent or higher amount of biogas with a lower energy requirement and GHG emission.

1.2 PROBLEM STATEMENT AND HYPOTHESES

Anaerobic digestion of sewage biosolids can effectively reduce excess sludge production and enable the recovery of valuable energy through biogas production. Operating anaerobic digesters at high mesophilic temperatures (30-37 °C), however, increases energy expenditure especially in cold climate countries such as Canada. In this context, the concept of low mesophilic anaerobic digestion (20 ºC) combining biosolids ozone pretreatment, was explored in with the objective of improving energy sustainability of the technology as well as lowering the capital investment and operational costs associated with it. Compared to pretreatment technologies such as the CAMBI thermal hydrolysis, which involves operation at high temperatures and pressure and requires complex installations, ozone pretreatment is not energy intensive and is simpler to implement. Additionally, ozone is a powerful oxidant, which can disrupt biosolids, destroy cellular components and solubilize organic materials to help overcome the rate-limiting step of hydrolysis and facilitate the process of anaerobic digestion.

Our hypothesis was that ozonation can increase the hydrolysis rate by solubilizing chemical oxygen demand (COD) and transforming non-biodegradable particulate organics to biodegradable substrates to enable the anaerobic digestion of WAS at low mesophilic temperature. An increase in sludge biodegradability can result in a shorter SRT in the digester and an enhanced biogas production (Rittmann & McCarty, 2001c). Beyond increasing the rate of biogas production, ozonation has also been shown to increase the ultimate degradability of WAS (Chu et al., 2009). Several key questions were addressed to verify this hypothesis, and they were answered through the following objectives.

1.3 RESEARCH OBJECTIVES

The objectives of this research project were as follows:

a) To demonstrate the feasibility of high-rate anaerobic digestion at low mesophilic temperature (20 °C) combined with ozone pretreatment to produce a biogas yield and biosolids reduction equivalent to or better than a conventional anaerobic digestion at 35 °C.

b) To explore the additional performance at 20 °C after ozone pretreatment of WAS by comparing kinetic parameters and phylogenetic diversity of archaeal and bacterial populations.

c) To optimize the biosolids reduction and methane production at 20 °C combined with ozone pretreatment of WAS.

d) To compare the plant-wide carbon footprint, energy performance and operational cost of anaerobic digestion systems at high mesophilic temperature (35 °C) and low mesophilic temperature (20 °C) with WAS ozonation.

1.4 THESIS ORGANIZATION

Following the introduction and literature review chapters, this thesis is structured into five chapters describing novel research followed by a chapter of general discussion and conclusions.

Chapter 3 lays the foundation of this research project where an initial hypothesis was formulated and eventually verified as the research unfolded. Three research questions were asked: a) Is high-rate anaerobic digestion feasible at low mesophilic temperature (20 °C) using ozone treatment of biosolids? b) If feasible, what are the performances of a combined anaerobic digestion-ozonation system at low mesophilic temperature (20 °C) as compared to conventional anaerobic digesters operated at 35 °C in terms of methane production and sludge destruction? c) What type of energy gain can be achieved with an anaerobic digester combining ozonation, and operated at low mesophilic temperature (20 °C) as compared to conventional anaerobic digestion at 35 °C? To provide preliminary answers to these questions, three anaerobic bench-top reactors were operated for 350 days. The performance of the reactors in terms of solids reduction and biogas production was evaluated. A simple energy balance was conducted to evaluate the potential energy gain or loss on two anaerobic digesters: 20 °C with sludge ozonation and 35 °C without sludge ozonation.

Chapter 4 builds on the findings of Chapter 3. This study investigated the effect of ozone treatment of WAS or anaerobic digestate on the yield and rate of methane production during anaerobic digestion at 35 °C and 20 °C. A Modified Gompertz model was used to predict methane production from anaerobic digestion of ozone-pretreated and untreated WAS or anaerobic digestate. The results provided insights on the kinetic parameters on WAS or digestate solubilization during ozone treatment. The phylogenetic diversity of archaeal and bacterial

populations of three bench-top anaerobic digesters at different temperatures (35 °C and 20 °C) and feeding composition (ozonated biosolids vs non-ozonated biosolids) was also studied. The specific goal was to determine the effect of temperature and feeding composition on the microbial community structure of the digesters. The microbial community structure was studied by sequencing PCR amplicons of 16S rRNA genes by the Illumina MiSeq300 technology.

Chapter 5 builds on the findings of Chapter 4 by further examining the hypothesis that ozonation of biosolids is feasible at low mesophilic temperature. The following research questions were investigated: a) Does ozonation treatment of influent WAS or recirculated anaerobic digestate affect sludge reduction and biogas production, and which of these is the optimal point of ozonation? b) What is the optimum SRT at which anaerobic digestion process performance is most sensitive to WAS/digestate ozonation? c) Does decoupling of SRT/hydraulic retention time (HRT) have an impact on biogas production and sludge reduction? d) What are the impacts of temperature (35ºC vs. 20 ºC) and ozonation (ozonated vs. non-ozonated substrates) on the archaeal and bacterial community composition and structure of the anaerobic digesters? To answer these questions, we operated four lab-scale anaerobic digesters: 20 °C fed with ozonated WAS and recirculated raw (untreated) anaerobic digestate, 20 °C fed with ozonated WAS and recirculated ozonated anaerobic digestate, 35 °C fed with raw WAS, and 35 °C fed with ozonated WAS and recirculated raw anaerobic digestate. To address knowledge gaps about changes in the microbial community of anaerobic digesters at different temperatures and feeding composition and their relationship to CH_4 production, the microbial community structure in the anaerobic digesters operating different temperature and feeding regimes was studied by sequencing 16S rRNA PCR amplicons.

Chapter 6 uses the findings of Chapter 5 to construct a plant-wide model including activated sludge as the main wastewater treatment process and an anaerobic digester as the sludge treatment process. Six different configurations of the anaerobic digestion process were studied: 20 °C fed with (1) ozonated WAS or (2) raw (untreated) WAS and 35 °C fed with (3) ozonated WAS or (4) raw WAS, (5) 35 °C fed with thermophilically (55 °C) hydrolyzed WAS [this represents the current configuration of the full-scale plant used in our case study] and 35 °C fed with ozonated and thermophilically hydrolyzed WAS. The outputs of the plant-wide model were used as the main inputs for energy balance performance, GHG emissions and carbon footprint analyses to evaluate

the sustainability of the different operational systems. The carbon footprint of each operational configuration and their associated GHG emissions were evaluated based on the Intergovernmental Panel on Climate Change (IPCC) Guidelines for National Greenhouse Gas Inventories (Eggleston et al., 2006). We identified GHG emissions from every step of the life cycle of the biosolids based on a "cradle-to-grave" analysis from the start of the treatment train until disposal at landfill or land application for agriculture. We also included in our inventory, the GHG emission associated with transport of the treated biosolids to the landfill or agricultural sites. The operational costs and benefits of each configuration were also investigated in this chapter.

Chapter 7: provides a general discussion of the salient findings of the thesis and general conclusions of this doctoral research.

1.5 CONTRIBUTION TO ORIGINAL KNOWLEDGE

Disposal of biosolids produced during wastewater treatment represents a serious environmental and economic problem, with waste biosolids handling and disposal alone representing up to 60% of the operational costs of biological wastewater treatment facilities (Liu, 2003). Anaerobic digestion of WAS is one of the most common processes used for biosolids reduction. However, the high proportion of slowly biodegradable and non-degradable particulate materials in WAS limits effectiveness of the technology and forces operation at temperatures above 30 °C with long retention time of 20-30 days (Bougrier et al., 2007; Mottet et al., 2013; Rittmann & McCarty, 2001c). Although a significant number of studies have been undertaken to overcome this rate-limiting step using various pretreatments (Carrere et al., 2010), no particular attention has been given to their potential in reducing the operational temperatures (20 °C or below) to conserve energy. At the beginning of this work, we hypothesised that ozonation can enhance the energetic, carbon footprint, and economic performance of anaerobic digestion of biosolids by increasing the degradability and biodegradation rate of the WAS, and by allowing the operation temperature to drop to low mesophilic range (~20 °C), while producing a biogas yield equivalent to or better than a conventional anaerobic digester operated at 35 °C. This hypothesis was investigated and developed with the following contributions.

a) **Demonstrated the feasibility of 20-day SRT anaerobic digestion at low mesophilic temperature (20 °C) in a system combining ozonation.** Previous studies showed that

the contribution of ozone treatment prior to mesophilic anaerobic digestion (35-37 °C) was to increase the digester's performance. In this thesis, it is shown that by lowering the temperature to 20 °C and using ozone treatment, the digesters also displayed better performance in terms of sludge reduction, biogas production, and overall energy performance than high-rate conventional anaerobic digestion at high mesophilic temperature (35 °C).

b) **Identified the kinetic parameters associated with the anaerobic digestion of ozonated WAS or anaerobic digestate at different operational temperatures.** Previous research studied the kinetic parameters for anaerobic digestion at mesophilic temperatures of 37 °C or higher. The current study identified the set of kinetic parameters applicable to methane production from anaerobic digestion of ozone-pretreated and untreated WAS or anaerobic digestate at low mesophilic temperature (20 ºC).

c) **Characterized the phylogenetic diversity of methanogenic microbial populations (archaeal and bacterial) of anaerobic digesters operated under different conditions (temperature and feed composition).** This is important since the microbial communities drive the anaerobic digestion process and production of biogas. Altering the operational conditions have an impact on the microbial community structure and composition and affect acetoclastic and hydrogenotrophic methanogenesis.

d) **Developed plant-wide models for anaerobic digestion with different configurations and evaluated their impacts on energy requirement, carbon footprint and cost of operation.** Plant-wide models represent useful engineering tools to designers, modelers, plant operators and decision-makers for designing, upgrading and improving the sustainability and efficiency of wastewater treatment systems. Based on the simulation results, integrating sludge ozonation with anaerobic digestion was found to impart benefits including feasibility at low mesophilic temperature (20 ºC), enhanced digester performance in terms of volatile suspended solids (VSS) reduction and biogas production, reduced carbon footprint, and lower operational cost, compared to conventional anaerobic digestion at 35 ºC.

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CHAPTER 2

Literature review

2.1 BIOSOLIDS MANAGEMENT

Wastewater treatment is an important component of the national efforts aimed at protecting public health, water resources and the environment. However, the treatment process generates tons of biosolids. Biosolids contain varying amounts of organic matter, metals, chemicals, and pathogens (Harrison et al., 2006). According to the Environmental Protection Agency (EPA, 2009), untreated biosolids constitute a hazard to human health and the environment. In 2009, the Canadian Council of Ministers of the Environment (CCME) developed the Canada-wide approach for the management of wastewater biosolids (CCME, 2009). The goal of the approach was to build a framework which can establish public confidence and protect the environment and human health. It outlines the beneficial use and management of municipal biosolids, sludge and treated septage as valuable sources of nutrients, organic matter and energy. Current legal biosolids disposal options include incineration, anaerobic digestion, landfilling, and land application to agricultural land, rangeland, or forests. In Canada, approximately 660,000 metric tons of dry stabilized biosolids are produced every year (CCME, 2009). About 41% of these biosolids are applied to land, 22% are incinerated and 17% are sent to landfill, with the remainder used in land reclamation and other uses. Land application has been increasing in recent years as many municipalities move away from incineration and landfill disposal due to environmental concerns. By comparison, the United States and Europe apply approximately 60% and 34%, respectively, of their biosolids to agricultural land. According to the US-EPA, biosolids meeting Class A or B microbiological requirements can be applied to agricultural lands (USEPA, 2003). Class A requirements specify a reduction of pathogen indicators such as fecal coliforms, *Salmonella* spp., enteric viruses and helminth ova, to essentially pathogen-free biosolids while Class B requirements indicate a relatively low concentrations of below 2×10^6 MPN/g TS or CFU/g TS of fecal coliforms with restricted land application for grazing animals and public access. Canadian guidelines follow the same regulations as those of US-EPA (CCME, 2010b).

In Quebec, 910, 000 tonnes of biosolids resulting from wastewater treatment are generated each year (Villeneuve & Dessureault, 2011). In the specific case of municipal biosolids, the Ministère du Développement Durable, de l'Environnement et des Parcs (MDDEP) estimated that in 2007, the majority of biosolids produced was incinerated (42%) or landfilled (31%) while only 25% of Quebec biosolids were beneficially used for soil conditioning via direct agricultural land

application (16%) or through composting (9%) (Villeneuve & Dessureault, 2011). At present, landfilling is the most common method for biosolids disposal in many countries due to its relatively low cost. Landfilling, however, is becoming increasingly difficult as a result of reduced land availability, increasing compliance costs, public opposition, and leachate and greenhouse gas (GHG) emissions (methane is a GHG which is 25× more potent than CO_2). To ensure that organic materials are managed in a sustainable manner and in order to help meet the objectives of the Climate Change Action Plan and the Quebec Energy Plan (Gouvernement du Québec, 2012; Québec, 2016), the provincial government has imposed a ban on the disposal of organic wastes by landfilling and incineration by 2022. In parallel to the ban imposition, Quebec has also set the goal of reusing 60% of biosolids by 2015 through bio-methanation, composting and spreading of residuals to fertilize land, which unfortunately has not been achieved. Various strategies for biosolids management, treatment and disposal recommend the conversion of biosolids to valuable materials and energy. Bio-methanation using anaerobic digestion has been commonly used to recover energy from biosolids.

2.2 ANAEROBIC DIGESTION

Anaerobic digestion is one of the most common processes used to stabilize organic matter in biosolids, and to decrease pathogen levels and odors (Dewil et al., 2007). Biosolids are highly putrescible and have to be stabilized prior to safe disposal in the environment (Nickel, 2007). Anaerobic digestion involves the biological conversion of organic matter to methane, carbon dioxide and biomass in the absence of oxygen. Typically, it proceeds in sequence through four general microbial steps which are mediated by a complex consortium of microorganisms intimately linked and working in a coordinated manner in an anaerobic food web (Fig. 2.1): (1) disintegration and hydrolysis, (2) acidogenesis, (3) syntrophic acetogenesis, and (4) methanogenesis (Grady et al., 2012).

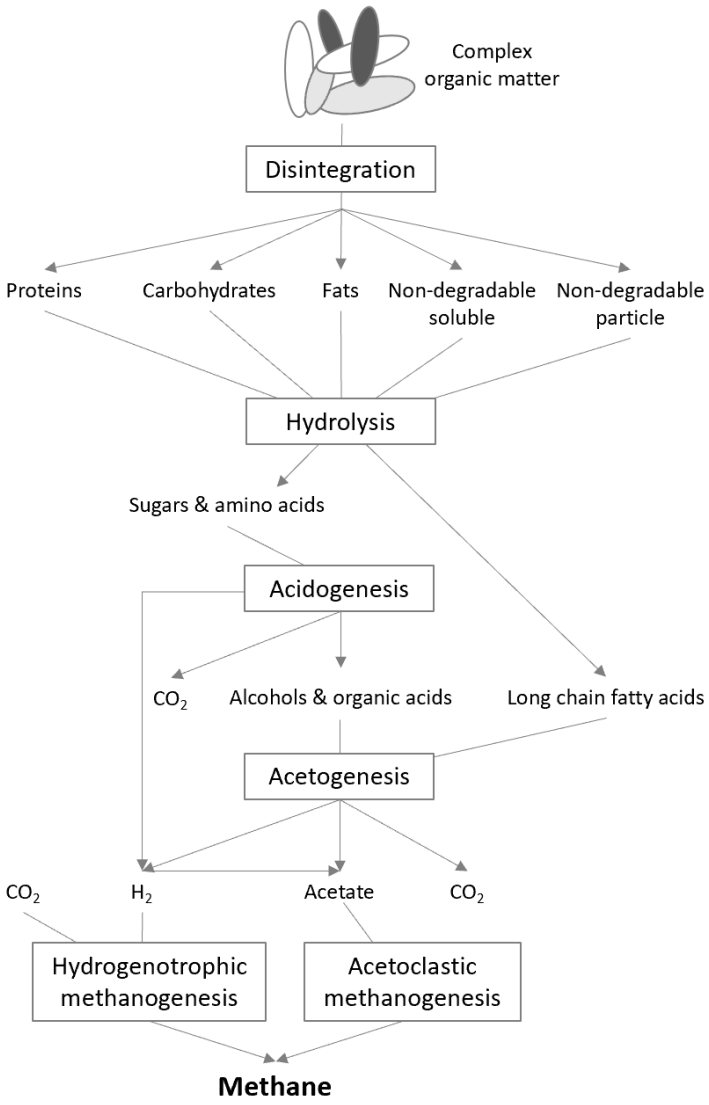

Fig. 2.1. Proposed metabolic pathway for methane production from anaerobic digestion of complex organic matter. Step 1: disintegration and hydrolysis of complex organic matter, Step 2: acidogenesis from sugars and amino acids, Step 3: acetogenesis from alcohols, volatile fatty acids (VFAs) and long chain fatty acids (LCFAs), and Step 4: methane production through acetoclastic and hydrogenotrophic methanogenesis (modified from Batstone and Jensen (2011)).

The first three steps of the anaerobic digestion process are performed by *Bacteria*. The first step involves the disintegration of complex organic particles into macromolecules and polymers such as proteins, polysaccharides and lipids followed by their hydrolysis mediated by extracellular enzymes to their monomers (amino acids, sugars and long chain fatty acids, respectively) (Batstone

& Jensen, 2011). Key bacterial genera involved in the hydrolytic process include *Clostridium*, *Cellulomonas*, *Bacteroides*, *Succinivibrio*, *Prevotella*, *Ruminococcus* and *Fibrobacter* among others (Gerardi, 2003). During the second step of acidogenesis, the amino acids and sugars are fermented into alcohols and short-chain carboxylic acids by acidogenic bacteria such as *Peptoccus*, *Clostridium*, and *Lactobacillus* (Sun et al., 2015), while the longer chain acids and alcohols (e.g., propionic and butyric acids) are further transformed to acetic acid, H_2 and CO_2 during the third step of acetogenesis (Grady et al., 2012). Studies have shown that acetogenesis is strictly linked to the partial pressure of H_2 (P_{H2}) and proceeds favorably when the P_{H2} is less than 10^{-4} atm (Grady et al., 2012). Consequently, acetogenesis requires a syntrophic association between the acetogens and hydrogenotrophic methanogens. Typical acetogenic genera include *Syntrophobacter*, *Syntrophus* and *Syntrophomonas* (Cai et al., 2016).

The final fourth step of anaerobic digestion involves methanogenesis during which electron equivalents that had accumulated in acetate and hydrogen through the previous reactions are accepted by different carbon moieties to produce methane (CH_4), the most reduced oxidation state (−4) of carbon (Rittmann & McCarty, 2001b). Methanogenesis is performed by a specialized group of obligate anaerobic microorganisms called methanogens, belonging to the *Archaeal* domain and phylum *Euryarchaeota*. This group is of special interest to the scientific community since it is the only group of microorganisms capable of methane production (Liu et al., 2008). The methanogens have been classified based on their specific substrate requirements. Acetoclastic methanogens such as *Methanosarcina* and *Methanosaeta* consume acetic acid and convert it into methane and carbon dioxide. Hydrogenotrophic methanogens such as *Methanobacterium* and *Methanoculleus* oxidize H_2 and reduce carbon dioxide to produce methane (Lu et al., 2015; Rittmann & McCarty, 2001b).

2.3 CONVENTIONAL OPERATION OF ANAEROBIC DIGESTERS AND ITS LIMITATION

Conventional anaerobic digesters are operated to reduce excess biosolids production and enable energy recovery (Chu et al., 2008). However, the application of this technology has limitations because waste activated sludge (WAS) contains a high proportion of slowly biodegradable particulate substrates as well as non-degradable particulate matter. Their biodegradation requires long retention times of 20-30 days at 35 °C (Rittmann & McCarty, 2001b). Despite this long SRT,

the non-degradable portion limits the volatile solids destruction to 30-50% (Mottet et al., 2013). The kinetic "bottleneck" of the anaerobic digestion system becomes the disintegration and hydrolysis of both insoluble organic material and high molecular weight compounds such as lipids, polysaccharides, proteins and nucleic acids (Batstone et al., 2009). At temperatures lower than 30 °C, the minimum required SRT increases rapidly with decreasing temperature, leading to large reactor volumes (Fig 2.2) (Buhr & Andrews, 1977; Rittmann & McCarty, 2001b). Thus, the reason for operating conventional anaerobic digesters at high mesophilic temperature of 30-37 °C is to accelerate the hydrolysis step and minimize the SRT.

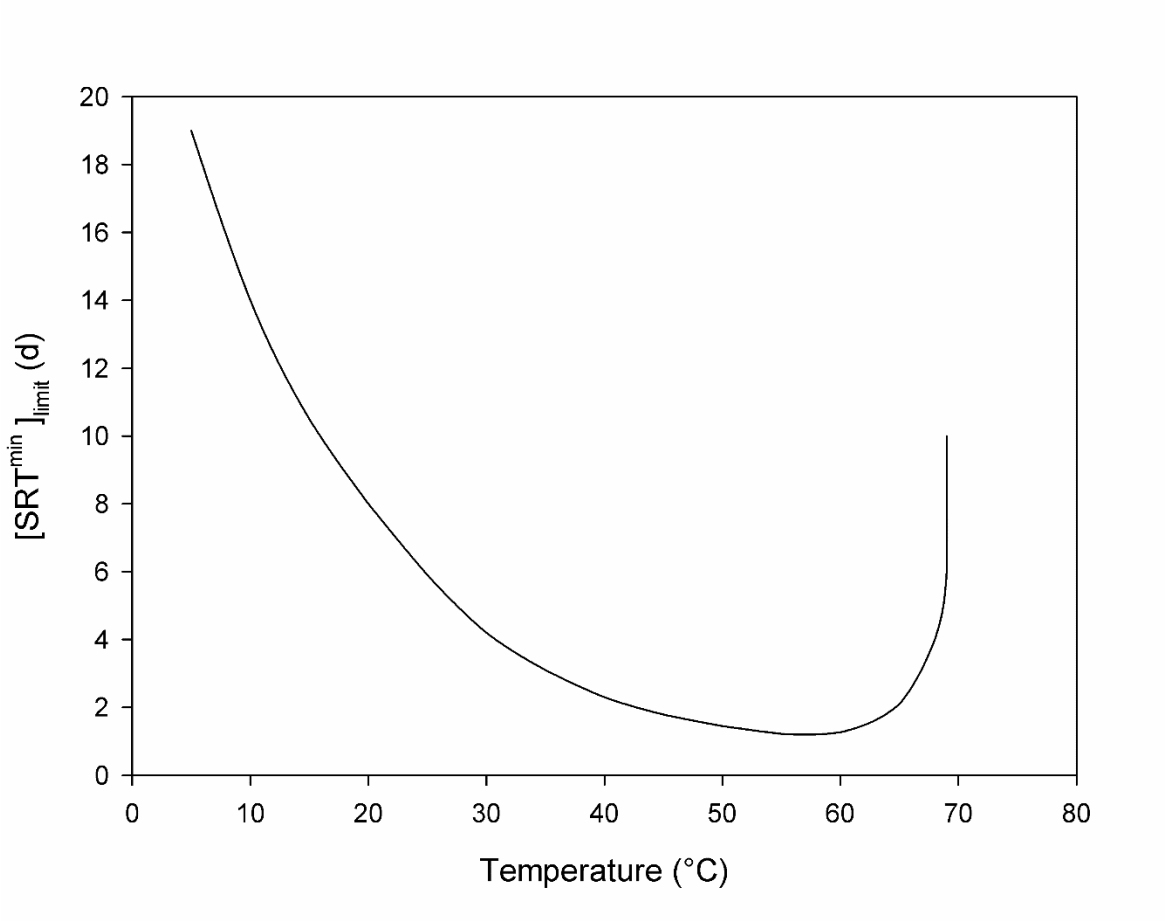

Fig.2.2. Solid retention time (SRT)-temperature profile for anaerobic digestion. Conventional anaerobic digesters are operated at 30-37 °C for absolute minimum SRT ranging between 3-4 days. By decreasing the temperature, the minimum SRT increases (Buhr & Andrews, 1977).

Studies have shown that mesophilic digestion is not very efficient in reducing biosolids with significant level of very slowly degradable or non-degradable particulate matter (Batstone & Jensen, 2011; Ekama et al., 2007). This problem is mainly seen when digesting sludge from activated sludge reactors operated at long SRTs or with sludge of industrial origin (Ekama et al., 2007). The efficiency of mesophilic anaerobic digestion with respect to both destruction of organic matter and methane production can be increased by physically segregating disintegration/hydrolysis/acidogenesis and methanogenesis into a two–stage digester system (Montañés Alonso et al., 2016; Pohland & Ghosh, 1971). The growth of methanogens is discouraged in the first reactor by maintaining the SRT at less than 5 days, which results in high production of volatile fatty acids (VFAs; also known as short chain carboxylic acids) and a low pH. The second reactor is maintained at high pH to favor the growth of methanogenic *archaea,* which utilize the VFAs to produce methane (Gonzalez-Martinez et al., 2016; Schmit & Ellis, 2001). Bolzonella et al. (2012) reported that the percentage organic matter destruction and biogas production were 50% more in a two-stage system as compared to a single-stage digester

In order to maintain an efficient hydrolysis of substrates, high temperature mesophilic and thermophilic anaerobic digestion systems have also been developed (Bolzonella et al., 2012). However, in both cases, the heat energy requirement is significant to sustain the anaerobic digestion process (Tchobanoglous & Burton, 1991). To the best of our knowledge, no attempt has been made so far to anaerobically digest complex substrates such as municipal biosolids at low mesophilic temperatures. Some authors have studied the application of low mesophilic temperatures (15-20 °C) for the anaerobic digestion of municipal wastewaters which are more biodegradable (Collins et al., 2005; Hill et al., 2001; Wu et al., 2017). The process has been shown to be feasible and stable with this type of substrate and at these temperatures but with long SRTs. Such low temperature anaerobic digestion has also been applied to the digestion of animal manure. Alvarez and Lidén (2009) used a mixture of llama, cow and sheep manure in an anaerobic digester operated at 18–25 °C and observed methane yields between 0.07–0.14 $m^3\ kg^{-1}\ VSS_{added}$. McKeown et al. (2009) have shown that the mesophilic inoculum can even adapt to psychrophilic conditions if operated under this temperature profile for an extended period, they obtained a methane yield of more than 4L of CH_4/d using acidified wastewaters.

2.4 PRE-TREATMENT METHODS

The disintegration and hydrolysis of complex organic substrates are recognized as the main rate-limiting steps during anaerobic digestion (Appels et al., 2008). Various pre-treatments such as thermal, mechanical and chemical methods have been applied to increase their rates. All these pre-treatments aim at disintegrating the biosolids, solubilizing organic materials and transforming non-degradable components to degradable ones for easy uptake by microorganisms (Carrere et al., 2010). Such strategy is interesting as it reduces long SRTs, digester volume and capital investment (Tiehm et al., 2001).

Thermal hydrolysis is performed at temperatures ranging between 160-180 °C and pressures in the range of 600-2,500 kPa (Bougrier et al., 2008; Sapkaite et al., 2017). All studies have reported a positive impact of thermal pre-treatment although the optimum conditions and magnitude of improvement in sludge degradability depend on the nature of the sludge (Gavala et al., 2003). Dwyer et al. (2008) applied thermal hydrolysis to different waste sludges and found that by increasing the treatment temperature to 150 °C an increase in sludge solubilization and in methane conversion was observed. Further increasing the temperature to 170-190 °C weakened biodegradability due to the formation of recalcitrant compounds such as melanoidins (high-molecular-weight heterogeneous polymers). Sapkaite et al. (2017) studied the thermal hydrolysis of activated sludge as a pre-treatment method between 130-180 °C and over 5-50 min. The results showed that methane production and solubility of solids were significantly affected by pre-treatment time and temperature. At high temperature and long hydrolysis times, an increase in the concentration of soluble COD and biogas production was observed.

Mechanical treatment involves several strategies to physically disintegrate the biosolids flocs and partly solubilize their contents. The use of colloid mills (with stationary and rotating discs), high-speed shakers (with grinding glass beads) or large-scale high-pressure homogenizers physically disrupts bacterial cells and releases their soluble contents (Muller & Pelletier, 1998). Ultrasonication is one of the most powerful methods to disintegrate biosolids (Cesaro & Belgiorno, 2013). During this process microbubbles are formed which violently collapse within a few microseconds to form cavitation. Successive compression and expansion of the fluid under the effect of ultrasonic waves induce implosions of the flocs leading to extreme localized temperatures (up to 1000 °C) and pressures (up to 500 bar) which initiate powerful hydro-mechanical shear

forces and highly reactive radicals (H· and ·OH). Although this technique can result in 100% cell disruption, the power consumption associated to it is extremely high (Le et al., 2015). Lizama et al. (2017) studied ultrasonic pre-treatment at a range of 5,000-35,000 kJ/kg TS (total solids) of WAS. The results show that a biogas production of 31.43% (219.5 mL/g VS) was achieved using ultrasonicated WAS compared to raw WAS.

Chemical pre-treatment is another option to enhance anaerobic digestion and includes acid, alkaline and oxidative hydrolysis (Carrere et al., 2010). Alkaline pre-treatment is relatively effective in sludge disintegration and solubilization. The most effective alkaline compounds in order of efficacy are NaOH > KOH > Mg $(OH)_2$ and Ca $(OH)_2$. Nevertheless, inhibition of anaerobic digestion may occur at high concentrations of Na^+ or K^+ (Carrere et al., 2010). Compared to thermal hydrolysis alkaline treatment is carried out at lower temperatures of 130-170 °C. However, extreme pH levels may require that the sludge be neutralized at the end of the treatment, which may limit its application. Hydrogen peroxide is one of the methods used for oxidative hydrolysis pre-treatment. The biogas production during anaerobic digestion was found to be enhanced by 16% by means of oxidation at 90 ◦C with 150 mmol/L H_2O_2 (Valo et al., 2004).

2.5 OZONE AS A PRE-TREATMENT METHOD

The most widely used oxidative method for sludge pre-treatment is ozonation (Cheng & Hong, 2013). Ozone is a powerful oxidant which can disrupt biosolids, destroy cellular components and solubilize organic materials. Thus, ozone can overcome the rate-limiting step of hydrolysis and facilitate the process of anaerobic digestion. In addition, due to the high oxidative power of ozone, studies have shown that applying an ozone dose between 0.13-0.67 mg O_3/mg TSS to biologically treated wastewater is sufficient for oxidizing many pharmaceutical and personal care products (PPCPs) by 90–99% (Huber et al., 2005). Most wastewater treatment facilities are not designed to remove pharmaceutical residues, and studies have shown that about 70% of these harmful products find their way in the treated effluent and around 30% end up in biosolids (Daughton, 2013). Pei et al. (2015) studied the acute biological toxicity of ozone pretreated pharmaceutical waste activated sludge by anaerobic digestion. The results showed that the acute toxicity of all the samples was reduced after anaerobic digestion compared to the untreated sludge. Hence, ozonation

is considered as one of the most promising pre-treatment technologies available in the wastewater treatment industry.

A significant number of studies have used ozonation on both aerobic activated sludge and anaerobic systems (Cheng & Hong, 2013; Chu et al., 2008; Chu et al., 2009; Isazadeh et al., 2015; Weemaes et al., 2000). Partial ozonation of return activated sludge (RAS) in conventional aerobic treatment systems has been shown to effectively solubilize COD, inactivate biomass and transform non-biodegradable particulate organics to biodegradable substrates, thereby reducing the production of excess sludge (Isazadeh et al., 2014). Activated sludge is composed of biomass linked by extracellular polymeric substances (EPS), inert materials and granules that form flocs (Zhang et al., 2009). Yeom et al. (2002) reported that the application of ozone at a rate of 0.1 g-O_3/g-TSS on raw sludge (comprising of 99.2% residuals with a negligible soluble fraction of 0.8%) resulted in a partitioning of the COD to 45% as intact solids, 24% as micro-particles, 26% as soluble COD and 5% as mineralized COD.

The concept of biosolids ozonation applied to aerobic activated sludge systems has been extended to anaerobic digestion of biosolids (Fig. 2.3a). Ozonation has been shown to improve sludge biodegradability and ultimately enhance anaerobic digestion and biogas production rate (Bougrier et al., 2006; Kianmehr et al., 2010; Weemaes et al., 2000; Yeom et al., 2002). This increase in the rate of biogas production reduces the SRT, in turn having an important impact on the sizing of digesters at the design stage since the primary design criterion for determining the digester volume is the retention time (Rittmann & McCarty, 2001b). Yasui et al. (2005) studied the anaerobic digestion of ozonated sludge at 37 °C and found that sludge dosed with 0.04 kg O_3/kg VSS not only increased the VSS degradation efficiency to 88% compared to non ozonated sludge, but also increased the methane production by 130% over the control digester. Studies have shown a 2-fold increase in the production of biogas when applying an ozone dose of 0.05-0.07 gO_3/gTSS (Goel et al., 2003a; Weemaes et al., 2000).

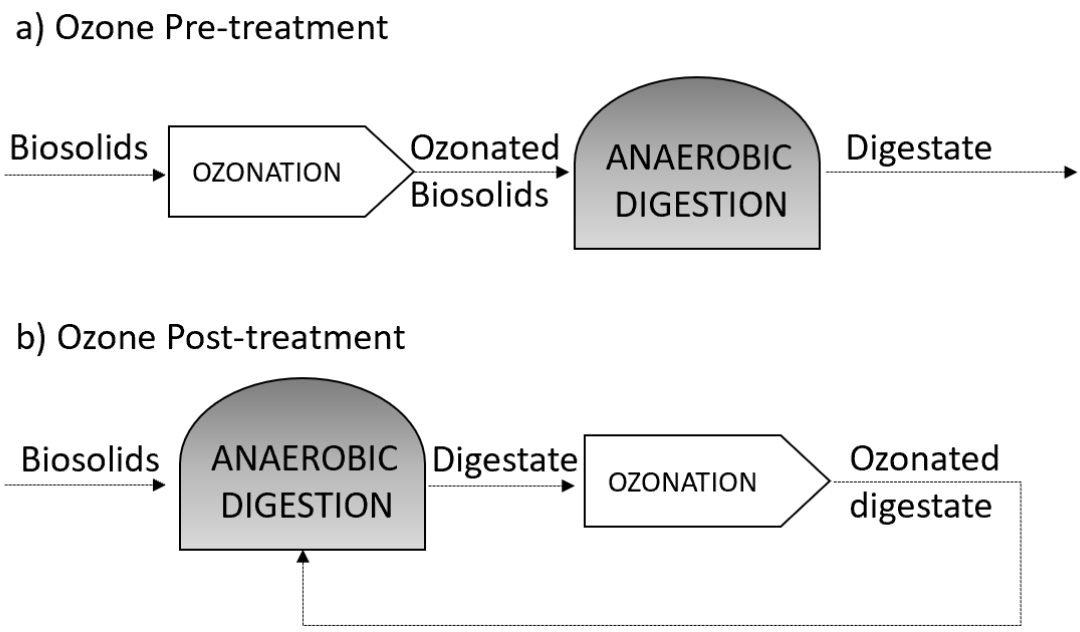

Fig. 2.3. Schematic diagram of a) anaerobic digestion with sludge pre-ozonation b) anaerobic digestion with sludge post-ozonation.

Ozone can also be used as a post-treatment option during anaerobic digestion of biosolids (Fig. 2.3b). Chacana et al. (2017c) studied the effect of ozone on primary and anaerobically digested sludge in batch experiments (using the un-acclimatized biomethane potential test) at high mesophilic temperature (35 °C). The results have shown that the soluble and biodegradable COD in the anaerobic digested sludge increased with an ozone dose of 90 mg O_3/g COD. The ozone post treatment enhanced the specific methane production and COD removal efficiency by 16% and 14%, respectively. Hence, these observations show the effects of ozone on the anaerobic digestion of biosolids at high mesophilic temperature (35 ºC) in improving digestion, organics removal and cumulative biogas production.

2.6 ANAEROBIC DIGESTION MODELS AND SYSTEM DESIGN

Anaerobic digestion models are important for designing bioreactors, optimizing operational conditions and predicting methane production, system stability and effluent quality (Angelidaki et al., 1999). Comprehensive models detailing the generation and degradation pathways of intermediates with associated interactions of microbial reactions, hydrodynamics, and mass transfer of substrates have been described by previous studies (Astals et al., 2013; Lauwers et al.,

2013; Vavilin et al., 2008). The most commonly adopted model is the Anaerobic Digestion Model No. 1 (ADM1) developed by the 'International Water Association (IWA) Task Group on Mathematical Modelling of Anaerobic Digestion Processes'. ADM1 simulates the degradation of complex substrates into carbohydrates, proteins, fats and inert compounds, which are further hydrolyzed to sugars, amino acids and long-chain fatty acids (Batstone et al., 2002). It simulates both biological reactions (disintegration and hydrolysis, growth and decay of microorganisms) as well as physiochemical reactions (solid-liquid, liquid-liquid and liquid-gas transfer). The model employs 26 state variables to describe the behavior of soluble and particulate components. All organic species and molecular hydrogen are described in terms of COD. Nitrogenous species and inorganic carbon species are described in terms of their molar concentrations. Soluble organics include the monomers of complex polymers (sugars, amino acids, LCFAs), volatile fatty acids (VFAs) (propionate, butyrate, valerate, acetate), hydrogen and methane. All biochemical extracellular steps are assumed to be first-order while intracellular reactions use Monod-type kinetics for substrate consumption and biomass production. In addition, ADM1 also takes into account any inhibition of the biological activity by pH, hydrogen and free ammonia (Blumensaat & Keller, 2005).

Hydrolysis is considered to be a key rate-limiting step in anaerobic digestion as complex particulate organic matter needs to be degraded prior to consumption and metabolism by the microorganisms (Vavilin et al., 2008). Vavilin et al. (2008) suggested that hydrolysis takes place in two steps: (1) bacterial colonization where hydrolytic bacteria cover the surface of solids, and (2) production of enzymes by the bacteria on or near the particle surface to degrade complex polymers into monomers. As described above, ozonation can be applied to enhance the degradability of biosolids and help to overcome this rate-limiting step. The interaction between ozone reactions and biological activities in aerobic treatment systems has been successfully modeled using IWA consensus models such as activated sludge models (ASM) to capture the conversions of COD pools (Isazadeh et al., 2014; Isazadeh et al., 2015; Manterola et al., 2007). Using these COD fractions as inputs, ASM–based models have successfully incorporated RAS-ozonation to describe the transformation and inactivation processes induced by ozone on biosolids (Wang et al., 2008). These ASM models integrating ozonation have also been calibrated and validated using appropriate model constants, kinetic rate coefficients and parameterized COD fractions. The model proposed by Isazadeh et al. (2014) provides a comprehensive and precise

approach to describe the action of ozone on biosolids. This model extends the Activated Sludge Model No. 3 (ASM3); it explicitly separates biomass inactivation and non-biomass transformation by ozone into two distinct processes and uses empirically derived constants generated from laboratory and pilot-scale studies. Previous findings from the combined ozonation and aerobic system modeling can provide valuable insights into how similar approaches can be applied to model anaerobic digesters fed with ozonated biosolids. So far, a comprehensive anaerobic digestion model combining sludge pre-treatment by ozonation has not been developed. However, the ADM1 platform has been modified for other pre-treatment methods such as anaerobic digestion of thermally pretreated WAS to include additional disintegration and hydrolysis parameters while keeping the other reactions (acidogenesis, acetogenesis and methanogenesis) strictly equivalent to those of the standard ADM1 (Ramirez et al., 2009). Recently, a study has focused on modifying the BioWin anaerobic digestion model to incorporate additional stoichiometric and kinetic parameters resulting from high-pressure thermal hydrolysis of WAS during anaerobic digestion (Jo et al., 2018). For the model development, a first-order decay process was implemented to reflect changes in the anaerobic biodegradability of the endogenous products through pre-treatment. Souza et al. (2013) successfully modeled the anaerobic digestion of autohydrolysis-pretreated secondary sludge using Biochemical Methane Potential (BMP) tests as a data source for hydrolysis parameter estimation. Similarly, Hai et al. (2014) modified the ADM to include chemical sludge pre-treatment using H_2O_2.

The lessons learnt from the effects of ozonation on activated sludge, and on previously modified ADM models incorporating sludge pre-treatment can be helpful in modeling a hybrid sludge ozonation-anaerobic digestion process for eventual system design, operation and optimization.

2.7 ENERGY BALANCE AND CARBON FOOTPRINT

Development of sustainable energy supply systems is drawing more and more attention for mitigating greenhouse gas (GHG) emissions (Amon et al., 2007). According to the Intergovernmental Panel on Climate Change (IPCC), it is crucial to implement energy sustainable solutions and deploy renewable energy technologies to curb and reverse the impact of GHG emissions and climate change (IPCC, 2014). This can be made feasible by understanding energy requirement and recovery from these systems through energy balances to render them more

sustainable. Understanding energy balances in wastewater treatment and anaerobic digestion systems may thus offer the dual advantage of energy substitution and climate change mitigation.

2.7.1 Energy balance analysis

In the energy inputs to energy balance models (EBMs) involve the energy required for heating the digesters, mixing the digester content and compensating for heat losses through the walls, floor, and cover of the digester vessel while energy outputs are the energy produced from methane and heat recovery from heat exchangers. Energy balances provide useful insights on the energy flow in anaerobic digestion systems. Puchajda and Oleszkiewicz (2008) developed comprehensive energy balances for mesophilic, single-stage thermophilic and two-stage thermophilic-mesophilic digestion systems. They showed that a single-stage thermophilic digester result an overall available energy of +135 GJ/d. The two-stage thermophilic-mesophilic digestion involved greater amount of available energy (+155 GJ/d) which was due to additional heat recovery between the digestion stages. Similar EBMs have been developed for single-stage and two-stage thermophilic anaerobic digestion of food waste (Xiao et al., 2018) and Fenton pre-treatment of anaerobic digestion of secondary sludge (Pilli et al., 2016). Ruggeri et al. (2015) developed comprehensive and detailed sustainable energy approaches and tested their application to hydrogen and methane production during anaerobic digestion. They systematically evaluated sustainability, energy return on investment (EROI) and energy payback time (EPT) of different anaerobic digestion configurations, and defined score matrices to rate the sustainability of the different digester systems. In general, EBMs of anaerobic digestion systems can provide important knowledge on their optimization, means to generate positive net energy yields, GHG mitigation, efficient energy conversion and profitability (EuropeanCommission, 2010).

2.7.2 Plant-wide modeling

Wastewater treatment operation and costs may vary considerably from one treatment facility to another, depending on the type of influent, treatment technology and required effluent quality (Olsson et al., 2014). Therefore, it is important to find optimum solutions for the design and operation of an entire wastewater treatment system, by investigating the mutual relationships among the different unit-process elements involved in the wastewater and sludge lines, since these frequently differ from the simple compilation of solutions achieved for the design or operation of

each unit-process element separately (Grau et al., 2007). Models analyzing the whole treatment facility, commonly referred to as plant-wide models, are highly desirable, and consider the dynamic description of all relevant processes in the wastewater and sludge treatment lines. Development of such integrated models, which include mutual relationships among the different unit-process elements, however, is not straightforward due to incompatibilities and different descriptions of the components and transformations in standard process models (Vanrolleghem et al., 2005; Wentzel et al., 2006). Plant-wide modeling has proved to be a useful tool for assessing and improving the energy efficiency of wastewater treatment systems. With increasing attention being paid to the water–energy–carbon nexus, biogas production from sewage sludge digestion is a subject of interest in both energy and wastewater domains. In many cases, a fraction of the energy generated from anaerobic digesters in the form of biogas is used for heating purposes, whilst the rest can be employed for other requirements after conversion to electrical power. Hence, the possibility of energy recovery from wastewater is a key opportunity to promote energy savings in view of reducing operating costs (EPA, 2015). According to Bozkurt et al. (2016), plant-wide models can effectively help in tuning and optimizing treatment operations, and to select the best option to meet the desired criteria in the wastewater treatment facility such as low-energy consumption. Implementing such approach not only leads to energy savings but also reduce operating costs and entails environmental benefits such as lower GHG emission. Various studies have modeled wastewater treatment systems and integrated energy balance to optimize the plant performance (Jeppsson et al., 2006; Nowak et al., 2015). Various advanced modeling software has been developed to include the analysis of wastewater treatment performance and it's energy consumption and efficiency such as BioWin, Simba 6, gPROMS, Waste-to-Energy (W2E) ...etc. (Pretel et al., 2016).

2.7.3 Carbon footprint

Municipal wastewater collection, treatment and disposal systems are important contributors of GHGs to the atmosphere, and form an important component in national GHG inventories of member countries adhering to the IPCC Guidelines (IPCC, 2006). Carbon footprint analyses have became important to estimate the amount of GHG emissions from wastewater sanitation sector (Foley et al., 2010; Genzowsky & Bolle, 2014). The carbon footprint represents the total set of GHG emissions directly or indirectly emitted by an activity or resulting from the different stages

of the life-cycle of a product. Direct emissions from wastewater treatment include methane (CH_4) and nitrous oxide (N_2O) that can be biologically produced in sewers, wastewater and sludge treatment infrastructures. Indirect GHG emissions occur mainly through the consumption of electricity, fossil fuels (including transportation), use of chemicals (e.g. phosphate precipitation and sludge dewatering), and disposal of sewage biosolids.

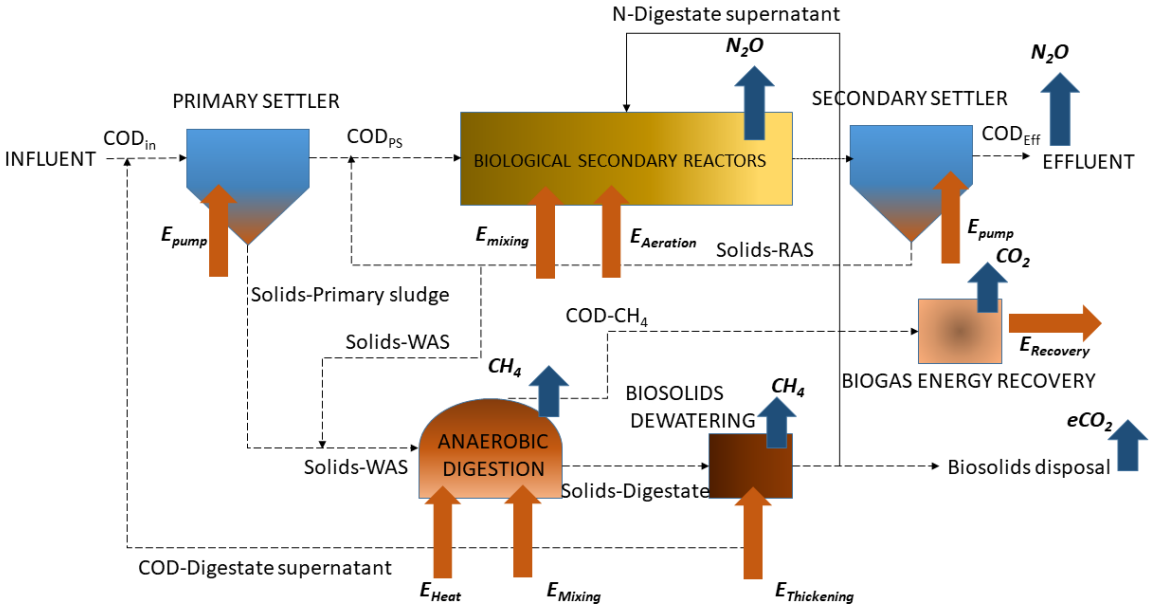

Fig.2.4. COD flow, energy requirement and GHG emission in a typical wastewater treatment system operating an anaerobic digester (Mannina et al., 2016). Arrows in dashed lines - COD flow; arrows in orange- energy requirements; arrows in blue – CH_4 emissions, N_2O emissions and equivalent CO_2 (eCO_2) emissions.

Recovery of energy in the form of biogas from anaerobic digesters represents an important means of reducing GHG emissions and mitigating the potential effects of global warming. According to the Canadian Biogas Association (CBA (2013), all biogas sources can reduce Canada's GHG emissions by 37.5 million tons eCO_2 per year, which is the equivalent to taking 7.5 million cars off the road. However, this can be achieved only by further exploiting and maximizing the recovery of biogas from anaerobic digestion systems (Puchajda & Oleszkiewicz, 2008). However, the knowledge on direct and indirect emissions from wastewater treatment systems is still inadequate

and warrants further research. Understanding the energy balance and GHG emissions will help to optimize design and operation of sustainable wastewater treatment infrastructures and improve their carbon footprint at local and global scale. Fig. 2.4 illustrates a typical plant-wide model showing the direct and indirect CO_2 emissions from biological and anaerobic digestion processes (Mannina et al., 2016).

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CHAPTER 3

Assessing energy benefits of operating anaerobic digesters at low temperature with solids pre-ozonation

Connecting text: Anaerobic digestion of sludge is one of the most widely used processes for biogas and energy production. Conventionally, anaerobic digesters are operated at 35 ºC to overcome the hydrolysis rate-limiting step. However, the energy expenditure for heating anaerobic digesters may be significant. In this first research chapter, by adding WAS ozonation as a pretreatment, the feasibility of operating anaerobic digesters at low mesophilic temperature (20 ºC) while keeping the SRT at 20 days was established. The 350-day experiment presented here compares results from three anaerobic reactor configurations. We demonstrated that, despite the low temperature of 20 ºC, WAS ozonation enhanced biosolids reduction and biogas production over the conventional 35 ºC without ozone treatment; however, the level of digestion at 20 ºC without WAS ozonation was minimal. Furthermore, an energy use/production model suggested the benefits of the low-mesophilic temperature operation with WAS ozonation configuration. This initial study was the justification for the expanded work reported in subsequent chapters.

The results of this research have been published as:

Zeinab Bakhshi, Shameem Jauffur, Dominic Frigon, Assessing energy benefits of operating anaerobic digesters at low temperature with solids pre-ozonation, Renewable Energy, Volume 115, 2018, Pages 1303-1311.

3.1 INTRODUCTION

Disposal of biosolids produced during wastewater treatment represents a serious environmental problem. Due to increased stringency of environmental standards governing the disposal of sewage sludge by different environmental protection agencies, their re-use in agriculture and for biogas production has gained increasing interest in recent years (Telles Benatti et al., 2002). In addition, sludge handling and disposal have important financial implications. Alone, it represents up to 50-60% of the operational costs of biological wastewater treatment facilities (Liu, 2003). Anaerobic digestion of waste activated sludge (WAS) is one of the most common processes used for biosolids reduction and can convert about 50% of organic matter present in sewage sludge into valuable methane biogas (Bougrier et al., 2007). Many European countries which have ratified the Kyoto Protocol, have introduced investment subsidies for the development of anaerobic digestion and biogas technologies. These subsidies, which may cover up to 40% of project costs, have supported the installation of hundreds of new biogas plants in Europe over the last 3-4 years (Grim et al., 2015). The renewed interest for anaerobic digestion is also reaching North America. In Quebec, for example, the imposition of the recent landfill disposal tax has triggered a dramatic increase in the disposal costs of sewage biosolids from $30/ton to approximately $100/ton (LeBlanc et al., 2008). Furthermore, according to the 2011 provincial regulation, landfilling of municipal biosolids will be banned by the year 2022 (Villeneuve & Dessureault, 2011). Subsequently, wastewater utilities are obliged to reduce their biosolids production, and are turning towards implementation of biosolids minimization technologies.

Anaerobic digestion is a multistep biological and chemical process consisting of four main sequential stages: disintegration/hydrolysis, acidogenesis, syntrophic acetogenesis, and hydrogenotrophic/acetoclastic methanogenesis (Batstone & Jensen, 2011). The first step, which is considered as rate-limiting, involves the disintegration of complex organic matter into polymers such as proteins, polysaccharides and lipids followed by their hydrolysis mediated by extracellular enzymes to their respective monomers (Batstone & Jensen, 2011). This limits the application of the technology since WAS contains a high proportion of slowly biodegradable particulate substrates as well as non-degradable particulate materials (Mottet et al., 2013). This is why mesophilic high-rate anaerobic digestion systems are typically operated at 35-37 °C to increase

the hydrolysis rate of slowly degradable organic matter. Still, their biodegradation requires a long retention time of 20-30 days at 35 °C (Rittmann & McCarty, 2001c).

Although temperatures higher than ambient are required in anaerobic digester to maintain a high rate of hydrolysis of substrates (Bolzonella et al., 2012), the heat energy requirement is significant to sustain the anaerobic digestion process (Tchobanoglous et al., 2003). Operation of anaerobic digesters at high mesophilic temperature range (35-37 °C) in Nordic climates such as the Canadian winter season implies a significant energy expenditure because the influent WAS generated by the aerobic treatment is at a much lower temperature (5-15 °C). According to Grant and Lin (1995), depending on the temperature of the matrix to be treated and the particular climatic conditions, it is not always practical to operate anaerobic digesters at the optimum temperature range because of the high energy requirements. Puchajda and Oleszkiewicz (2008) estimated that the operation of a single stage anaerobic digester at 35 °C with solids retention time (SRT) of 20 days would require an energy expenditure of about 34% of the biogas produced to heat up the reactor. Thus, operating anaerobic digesters at lower temperature would require lower energy expenditure. However, in practice, such low-temperature operation is not feasible due to the low rate of anaerobic digestion and biogas production.

Oxidative treatment of sludge using ozone is a promising technology to reduce excess sludge by breaking down biomass and non-biodegradable organic constituents in sludge making them bioavailable (Chu et al., 2009). According to Isazadeh et al. (2014), ozone can solubilize particles, inactivate biomass and transform non-biodegradable particulate organics into biodegradable substrates. Pre-treatment with ozone has been shown to increase sludge biodegradability leading to a shorter SRT in digesters operated at 35-37 ºC, and an enhanced biogas production (Rittmann & McCarty, 2001c). Goel et al. (2003b) found that a pre-treatment of biosolids with ozone (0.07 g O_3/g VSS_{in}) led to a significant improvement in anaerobic degradability, increasing the volatile suspended solid (VSS) destruction efficiency during anaerobic digestion at 35 °C to 60% from 31% for the control anaerobic digester, and increasing the biogas production by two-fold. Similarly, Weemaes et al. (2000) found a 2-fold increase in the production of biogas with an ozone dose of 0.07 g O_3/g VSS_{in} (0.1 g O_3/g COD_{in}) at a temperature of 35 °C and an SRT of 33 days.

Based on the above, the current study hypothesized that ozonation can increase the disintegration/hydrolysis rate making it feasible to operate at low mesophilic temperature (20 °C)

instead of the conventional anaerobic digester operated at 35 °C while maintaining similar SRTs. Furthermore, it is hypothesized that ozonation will increase the biodegradability of non-degradable VSS fractions and enhance the extent of VSS destruction. If such combined results from ozonation occurred, it will not only provide a technology to reduce excess sludge production but will also offer an operational strategy to increase the production of biogas and possibly the net energy gain by anaerobic digestion. Resultant from these hypotheses, the following questions were addressed in the current study:

1. Is high-rate anaerobic digestion feasible at low mesophilic temperature (20 °C) using ozone treatment of biosolids?
2. If feasible, what are the performances of a combined anaerobic digestion-ozonation system at low mesophilic temperature (20 °C) as compared to conventional anaerobic digesters operated at 35 °C in terms of methane production, and sludge destruction?

What type of energy gain can be achieved with an anaerobic digester combining ozonation, and operated at low mesophilic temperature (20 °C) as compared to conventional anaerobic digestion at 35 °C?

3.2 MATERIALS AND METHODS

3.2.1 Reactor operation

Three bench-top semi continuous anaerobic sequencing batch reactors (ASBRs) with a total and working volume of 1L and 0.8 L, respectively were operated for 350 days with a solids retention time (SRT) and hydraulic residence time (HRT) of 20 days (Figure 3.1). The reactors were fed daily with 40 mL of WAS. Each reactor comprised of a sealed vessel maintained in a water-bath and was agitated by means of magnetic stirrers. For the reactor start-up, anaerobic sludge from a full-scale digester, located at St-Hyacinthe (Quebec, Canada) was used to inoculate the benchtop digesters. The reactors were fed daily with waste activated sludge (WAS) collected bi-weekly from the LaPrairie wastewater treatment facility (Quebec, Canada). The physicochemical characteristics of the inoculum and feed are shown in Table S3.1. One of the reactors, referred to as the test reactor, was operated at 20 °C, and fed with ozonated WAS. Another reactor was used as a positive control reactor, and operated at the conventional temperature of 35 °C and fed with raw WAS. A third reactor was operated as a negative control reactor, at 20 °C and fed with raw WAS. The operational conditions and feeding regime of each reactor are summarized in Table 1. The three

reactors were operated for 350 days. During Phase I, the reactors were operated for 181 days to reach steady-state based on operational conditions described in Table 3.1. It is generally accepted that a reactor system requires at least three (3) SRTs to reach steady-state starting from the point of inoculation (Liao et al., 2006; Massé et al., 2006). The ASBRs were operated for 181 days (i.e. 3 times the required 3SRTs) to ensure that they completely reached steady-state prior to starting Phase II of the experimental run. During Phase II the contents of the three (3) reactors were mixed on Day 182, aliquoted back to each reactor and re-operated for 168 days based on conditions described in Table 3.1 to show reproducibility of Phase I. After every feeding event, the reactors were sparged with 100% nitrogen gas for 10 minutes at a rate of 1 L/min and kept isolated from the atmosphere. Each reactor was connected to a 1-L Tedlar® bag to collect biogas.

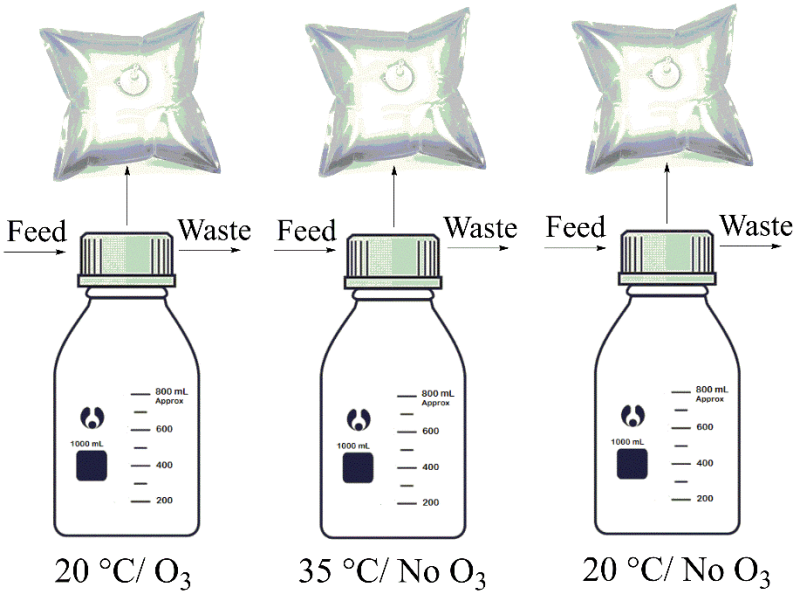

Figure 3.1. Three anaerobic sequencing batch reactors (ASBRs) operated at low (20 °C) & high (35 °C) temperatures, and under non-ozonated (No O_3) & ozonated (O_3) conditions

Table 3.1 Operational conditions for the anaerobic digesters

Reactor	**20°C/O_3[a]**	**35°C/No O_3[b]**	**20°C/No O_3[c]**
Description	Test reactor	Positive control	Negative control
Temperature (°C)	20	35	20
Solids retention time (SRT) (d)	20	20	20
Hydraulic retention time (HRT) (d)	20	20	20
Feed	WAS-O_3[d]	WAS[e]	WAS

Note:[a] 20°C/O_3 = Reactor operated with ozonated sludge; [b] 35°C/No O_3 = Reactor operated at 35 ºC with non-ozonated sludge; [c]20°C/No O_3= Reactor operated at 20 ºC with non-ozonated sludge; [d]WAS-O_3 = Ozonated waste activated sludge; [e]WAS = Waste activated sludge

3.2.2 Ozone treatment

Ozonation of WAS was performed in a 2-L laboratory-scale conical glass ozone contactor vessel equipped with two inlet ports fitted with atomizer nozzles supplying ozone at a pre-determined rate. The reactor content was continuously mixed by a magnetic stirrer during the ozonation to enhance gas transfer and prevent foaming. The ozone was generated using Ultra High Purity 4.3 oxygen (Praxair, Mississauga, Ontario) by an ozone generator (Ozomax, model OZO 3VTTL, Canton de Shefford, QC). The ozone dosage was determined by measuring the ozone concentrations in the feed and vent gas streams using an online Mini-HiCon O_3 Analyzer (INUSA Inc. Norwood, MA, USA). The optimum dose to ozonate the WAS was determined by chemical oxygen demand (COD) solubilization experiments, in which 1L of freshly collected WAS from the LaPrairie wastewater treatment facility was subjected to different ozone concentrations. The latter was controlled based on exposure time, and the corresponding soluble COD and volatile suspended solids (VSS) concentrations in the resulting homogenates were measured. COD solubilization efficiency was measured by plotting measured soluble COD versus ozone doses. WAS ozonated with an optimally determined ozone dose as established by the COD solubilization experiments, was used as feed for the test reactor.

3.2.3 Sampling and analytical methods

To assess the sludge degradability, grab digestate samples were collected twice weekly to determine the total, soluble and particulate COD fractions (method 5220D) (Rice et al., 2017). For measurement of the soluble COD concentration, the digestate was centrifuged at 4,000×g using the Sorvall™ ST 16 Centrifuge (Thermo Fisher, USA) for 20 min followed by filtration of the

resulting supernatant using 0.45-µm syringe filters. The level of total suspended solids (TSS) and volatile suspended solids (VSS) of the digester content was measured using method 2540D ((Rice et al., 2017). The concentration of ammonium (NH_4^+) was determined based on the Berthelot method involving the reaction of NH_4^+ as monochloramine with hypochlorite and phenol at a pH of 13 (Rhine et al., 1998). The test was performed by microplate assays with colorimetric measurements using the SPECTRAmax® microplate spectrophotometer (CA, USA). The spectrophotometer was operated using the SOFTmax® PRO software. Since reactor failure can occur due to acidification as a result of volatile fatty acids (VFAs) build-up (Appels et al., 2008), the level of VFAs in the reactor system was monitored using high-performance liquid chromatography (HPLC) (Thermo Scientific, UHPLC Dionex Ultimate 3000 MS LTQ XL, USA) equipped with an Eurokat H (300 × 8 mm) column. Sulfuric acid of 0.005 M was used as the mobile phase in the UHPLC. HPLC was operated under the following conditions: 30 ºC column temperature, 1 mL/min flow rate, pressure at 80 bar and 20 µl injection volume. Detection was performed by UV absorption at 210 nm. Biogas accumulated volume, to calculate the production rate, was measured by water displacement (Siegert & Banks, 2005). Biogas samples were analyzed using high-resolution gas chromatography with flame ionisation detector (GC-FID) (Agilent 6890, Agilent Technologies, USA) equipped with a GS-Q plot column (0.53 mm x 30 m, Agilent Technologies, USA). Samples were injected at 250 ºC injector temperature and oven temperature of 65 ºC. Nitrogen was used as the carrier gas with a flow rate of 30 mL/min.

3.2.4 Energy balance analysis

Energy balance models (EBMs) were developed, taking into account all energy quantities associated with the operational configurations of the anaerobic digestion system and were evaluated in energy units. Analogical EBMs showing energy demands and requirements for two different anaerobic digestion systems operated at 35 ºC with raw WAS and at 20 ºC with solids ozonation were evaluated. The different energy components of the model and the equations used to compute them are shown in Table 3.2. These include energy required to heat up the digester (E_{req}), energy produced from the biogas (E_{CH4}) which was estimated from the actual methane production (V_{CH4}) (Puchajda & Oleszkiewicz, 2008), electrical energy used to run the digesters in terms of mixing the digestate content (E_{mix}) which was estimated based on the method of Ruggeri et al. (2015), heat recovery (E_{rec}) resulting from a sludge heat exchanger used to capture heat energy from the sanitized or digested sludge leaving the digester tank (Puchajda & Oleszkiewicz,

2008), and energy required for ozonation which was evaluated based on the amount of ozone dosed per gram of solids (E_{O3}). Any difference between the operational temperature and the ambient air temperature resulted in heat loss (E_{loss}). The heat loss (E_{loss}) was determined by the product of the areas of the digester chamber (i.e. the side walls, floor and cover) and as the difference in temperature of the digester (T_{dig}) and ambient temperature (T_{air}) and their respective heat transfer coefficients (h_i) based on Tchobanoglous and Burton (1991) (Table 3.2, Equation (3.6) and (3.7), respectively). The net energy production (E_{net}) was computed based on the difference between the energy produced (E_{CH4} and E_{rec}) and the energy expended (E_{req}, E_{loss}, E_{mix} and E_{O3}) during the anaerobic digestion process.

To estimate the heat requirement, the flow of WAS to the digester was assumed to be 1,000 m^3/d, the temperature of incoming sludge (T_{in}) at 10 °C and the temperature inside the digester ($T_{digester}$) at 20 or 35 °C. To determine the heat loss from the system, the heat transfer coefficient for the walls, floor and cover of the digester were taken as 0.054, 0.025 and 0.103 MJ/m^2 °C, respectively (Tchobanoglous & Burton, 1991). The temperature of the ambient air (T_{air}) and the ground (T_{ground}) was assumed to be at 10 °C based on Tchobanoglous et al. (2003). It was deemed more appropriate to perform the energy balance study based on the average North American annual temperature of 10 °C to gain an appreciable order of magnitude of the resulting outcome regarding the effect of ozonation on anaerobic digestion since the heat requirements are highest during the winter period. On the other hand, recoverable energy comprised of energy produced from biogas and the heat recovered through heat exchangers. The energy obtained from biogas was estimated based on data generated from the laboratory-scale benchtop anaerobic digesters described above. The production of ozone for sludge ozonation requires electrical energy and a value of 12.5 kWh/ kg O_3 was used for estimating the amount of energy required for ozone production (Chu et al., 2009). The energy associated with heat requirement and ozone generation was converted to the equivalent biogas energy input to assess how much biogas energy is required to heat up the system and produce ozone. The following assumptions were made to estimate the amount of energy from biogas: sludge flow of 1,000 m^3/d, sludge density of 1,000 kg/m^3 at 4 °C (Tchobanoglous et al., 2003), total solids in sludge of 3%, ratio of volatile solids to total solids in sludge of 0.7 (Puchajda & Oleszkiewicz, 2008), retention time of sludge in the digester of 20 days, biogas to electricity efficiency of 35% (Mudhoo, 2012) and thermal heat efficiency of 70% (Darrow et al., 2015).

Table 3.2. Components of energy flow model in anaerobic digesters

Energy component	Mathematical equation	
Heat requirement	$E_{req} = Q\,(T_{dig} - T_{In})\rho_{sludge} \cdot c_{sludge}/\eta$	(3.1)
Energy from biogas	$E_{CH_4} = V_{CH_4} \times 37\ GJ/m^3$	(3.2)
Electrical energy	$E_{mix} = P_{wmix} \cdot t_{mix} \,/\, \eta_{el}$	(3.3)
Energy for ozone	$E_{O3} = O_3\ dose \times\ m_{solids} \times\ Elec_{O_3}/\eta el$	(3.4)
Heat recovery	$E_{rec} = Q\,(T_{exc-hot} - T_{exc-cold})\rho_{sludge} \cdot c_{sludge} \cdot \eta$	(3.5)
Heat losses	$E_{loss} = (E_{loss,wall} + E_{loss,floor} + E_{loss,cover})/\eta$	(3.6)
	$E_{loss,i} = A_i\,(T_{dig} - T_{Air\ or\ ground})\,h_i$	(3.7)

$Elec_{O3}$=12.5 kWh/ kg O_3; h_i = wall=0.054, floor=0.025 and cover=0.103 MJ/m^2 °C; Q = 1,000 m^3/d; T_{dig} = 35 or 20 °C; T_{in} = 10 °C; $T_{exc\text{-}hot}$ = 35 or 20 °C; $T_{exc\text{-}cold}$ = 10 °C; $T_{Air\ or\ ground}$=10 °C; ρ_{sludge} = 1,000 kg/m^3; c_{sludge} = 4,200 J/kg °C; η_{el} = 0.35; η = 0.7

Finally, to assess the sustainability of the two anaerobic digestion systems (35 ºC fed with raw WAS versus 20 ºC fed with ozonated WAS), the Energy Sustainability Index (*ESI*) as described by Ruggeri et al. (2015) was used, which takes into account the total amount of energy produced as biogas, and the amount of energy expended to heat-up the digester from ambient temperature to the operational temperature, and was computed using equation 3.8.

$$ESI = \frac{E_P}{E_C} \qquad (3.8)$$

Where E_p is the total energy produced (GJ/d) and E_c is the total energy consumed in thermal terms (GJ/d). An *ESI* < 1 means that the anaerobic digestion system is not energetically sustainable; an *ESI* > 1 means that the process is sustainable in thermal terms, and an *ESI* ~ 1 means the process is questionable (Malave et al., 2015).

3.2.5 Statistical analysis

Paired-measurement randomized complete block design (RCBD) ANOVA was performed for statistical analysis. Phase I and II were considered as independent blocks in the ANOVA. The last 60 days (i.e., steady-state periods) of each phase were subdivided into 3 time-periods of 20 days that were considered independent because the SRT of the reactors was 20 days; the average measurements from these periods were the basic observations for the statistical analyses. The observations were paired because the quality of the influent WAS may vary over time, but remain the same for the two treatments during each time-periods. The paired-observation RCBD was

implemented in Microsoft Excel 2016 using coding variables for blocks and computing the ANOVA with the linear regression procedure of the data analysis extension. The results of the RCBD ANOVA is depicted in Table S3.2.

3.3 RESULTS AND DISCUSSION

3.3.1 Biosolids reduction

The efficiency of digestion was evaluated based on the reduction in biosolids concentrations which were measured twice weekly. Ozone utilization was evaluated by difference in the residual ozone concentrations in the gaseous flow from the contactor vessel empty or containing WAS. Increasing the ozone dose resulted in an increase in the soluble COD concentration as shown in Figure S3.1. The solubilisation curve comprised of a linear portion where the ozone dose was directly proportional to the release of soluble COD and a plateau portion where no further increase in soluble COD was observed despite an increase in ozone dose. Hence, the optimum ozone dose was defined as the highest dose on the linear portion of the graph just before it reached the plateau. Consequently, the optimum ozone dose applied to the WAS entering the test reactor was determined to be 200 mg/L (or 0.02 g O_3/g VSS_{in} since the influent VSS concentration was 10,000 mg/L) (Figure S3.1).

WAS exposed to the optimum ozone dose was fed the test reactor on a daily basis. Results showed that both TSS and VSS reduction efficiencies were significantly better for the test reactor operated at 20 ºC and fed with ozonated WAS than for the positive control reactor operated at 35 ºC and the negative control reactor operated at 20 ºC, both fed with untreated WAS. Analyzing Phase I operation data showed that the test reactor (20 °C/O_3) reached steady-state (at least 3 SRTs having constant VSS concentration, biogas production rate and sCOD concentration) after 52 days of operation, while the positive control reactor (35 °C) reached steady-state after 40 days of operation (Figure 3.2). In Phase II, these start-up times before steady-state were reduced to 47 and 26 days, respectively. In both phases, negative control reactor (20 °C) took somewhat longer than the other two reactors to attain steady-state. Once at steady-state, the average feed VSS concentration of 9,882±125 (± standard error as in Table 3.3) mg/L was reduced to 3,782±78 (61±3% reduction) in the test reactor, 5,525±80 (44±2% reduction) in the positive control reactor, and 8,535±87 (14±3% reduction) in the negative control rector. Therefore, the ozone treatment of the WAS with

anaerobic digestion at 20 ºC resulted in a statistically significant higher reduction in average VSS by 39±3% and 3.36 times greater reduction as compared to the positive (35 ºC) and negative (20 ºC) controls, respectively (statistical test: paired-observation RCBD Table S3.2).

The biosolids destruction level was better for anaerobic digestion of ozonated biosolids at a low mesophilic temperature (20 °C) than for the conventional anaerobic digestion conducted at 35 °C. Such additional reduction in biosolids production is highly desirable, considering that sludge disposal poses a threat to the environment and requires significant financial outlay for both capital and operational purposes. Thus, the use of ozonation as WAS pre-treatment attracted the attention of a number of previous studies that have shown that WAS ozonation effectively increases the VSS degradation efficiency by anaerobic digestion at 37 ºC while keeping the retention times constant. For instance, Yasui et al. (2005) were able to increase VSS destruction by 88% by dosing sludge with 0.04 g O_3/g VSS_{in}, and Goel et al. (2003b) observed an increase in VSS destruction from 31% (control) to 60% when treating with 0.067 g O_3/g VSS_{in}. These results can be explained by two mechanisms. First, hydrolysis kinetics could be enhanced. The sludge flocs are composed of biomass linked by extracellular polymeric substances (EPS), non-degradable and degradable volatile solids, and non-volatile materials (Zhang et al., 2009). Ozone has been shown to disrupt sludge flocs and transform COD into different pools. Yeom et al. (2002) reported that the exposure of WAS to 0.1 g O_3/ g TSS partitioned COD into the intact solids (45%), micro-particles (24%), soluble COD (26%), and mineralized COD (5%); by breaking up particles and solubilizing COD, ozone provides a greater access of the biomass to the particulate substrates and enhances hydrolysis. Second, non-degradable VSS fraction is in part rendered degradable, which was demonstrated by Isazadeh et al. (2014). Thus, ozonation could further promote the degradation of WAS. From these considerations, ozone seemed to enhance sludge hydrolysis, which, in our study, allowed anaerobic digestion at lower mesophilic temperature (20 ºC instead of 35 ºC) while maintaining a 20-day SRT similar to high rate mesophilic digestion. Ozone also enhances biodegradability of the WAS, which increases the VSS destruction and biogas production. These benefits on the anaerobic digestion process can be achieved at low mesophilic temperature (20 ºC), without any requirement to supply additional thermal energy to heat up the digester to 37 ºC. It is shown that this approach is feasible, and it yields better results in terms of sludge reduction than the conventional anaerobic digestion at high mesophilic temperatures (35-37 ºC).

To show reproducibility of the data, the contents of the three digesters were combined, homogenously mixed on Day 182, and aliquoted back to the respective digesters. The digesters were operated under the same conditions as described in Table 3.1. The results mirrored those of the first phase of the experiment showing that the profile generated did not arise from experimental bias, but were effectively governed by the operational conditions and feeding regimes (Figure 3.2a). Similar to the first phase of the experiment (Phase I), a higher VSS destruction (60%) was observed for the test reactor (20 °C with ozonation) during Phase II than for the positive control (43%) and negative control (13%). This shows that ozone treatment of WAS prior to anaerobic digestion at low temperature represents a better option than conventional anaerobic digestion at high mesophilic temperature (35-37 ºC) in reducing excess biosolids production.

3.3.2 Biogas production

Measurement of biogas production showed that the test reactor operated at 20 ºC with ozonated WAS feed exhibited a statistically significant (paired-observation RCBD, Table S3.2) ~10% higher biogas production rate than the positive control reactor operated at 35 ºC with untreated feed (with an average for Day 250-350 of 990±15 and 900±6 mL/week for the test and control reactors, respectively) (Figure 3.2b). The negative control produced a very low level of biogas with only 40 mL/week. The biogas was composed of 54–62% methane, 35–40% CO_2, and 1–2% miscellaneous gases, which did not show significant variation between the test and the positive control reactor (35 ºC).

Specific methane production (SMP) was calculated based on two approaches: (i) by dividing the net volume of methane produced by the amount of initial VSS in the reactors (SMP_{VSSin}), and (ii) by dividing the net volume of methane produced by the amount of VSS destroyed in the reactors ($SMP_{VSSdestroyed}$). For the first case, at steady state, the test reactor reached an average SMP_{VSSin} of 232 mL CH_4/g VSS_{in} while the positive control achieved an average SMP_{VSSin} of 200 mL CH_4/g VSS_{in}. This shows that operation of the anaerobic digester with ozonated WAS at 20 ºC resulted in a better performance in comparison to the conventional anaerobic digestion at 35 ºC. Such approach seems more advantageous since the digestion is performed at ambient temperature without any supply of additional thermal energy. Based on the SMP computed using the amount of VSS destroyed, the test reactor resulted in an average $SMP_{VSSdestroyed}$ of 376 mL CH_4/g $VSS_{destroyed}$ while the positive control showed a higher $SMP_{VSSdestroyed}$ of 455 mL CH_4/g

$VSS_{destroyed}$. Although the biogas production rate and the amount of VSS destroyed were higher for the test reactor, a lower $SMP_{VSSdestroyed}$ shows that not all destroyed VSS was converted to methane. The lower conversion level of VSS to methane in the test reactor was due to the accumulation of volatile fatty acids (VFA) as shown in Figure 3.2d unlike for the positive reactor where no such accumulation of VFA was observed.

The higher transformation kinetics at 20 °C with sludge ozonation corresponds to observations by other groups for digesters at higher temperatures. Previous studies have reported the effect of ozone in enhancing sludge biodegradability and biogas production in anaerobic digestion at 37 ºC (Weemaes et al., 2000; Yeom et al., 2002). Yasui et al. (2005) studied the anaerobic digestion of ozonated sludge at 37 °C. In their experiment, sludge dosed with 0.04 g O_3/g VSS_{in} produced methane at a rate 130% higher than that of the control digester. This is beneficial since ozonation can also enable lowering the operational SRT. Bougrier et al. (2007) observed that an ozone dose of 0.14-0.22 g O_3/g VSS_{in} reduces the time to produce the same amount of biogas by anaerobic digestion at 37 °C, which, in their experiment, went from 24 days for the raw sludge to only 15 days for the ozonated sludge. Although the concept of solids ozonation has been applied to anaerobic digestion at 37 ºC, such an approach has not been previously tested at low temperature (20 ºC). By enhancing the biodegradability of the WAS, ozone enabled a higher production of biogas at low temperature (20 °C) as it was observed for previous experiments at 35-37 ºC. The current study specifically demonstrated that ozonation can effectively compensate for the slow hydrolysis of particulate substrate COD at low temperature (20 ºC) to overcome the rate-limiting step in anaerobic digestion, resulting in digestion kinetics equal or higher than at 35-37 ºC without ozonation. The combined effect of higher degradability and digestion kinetics resulted in a better digester performance at 20 ºC with ozonation as compared to conventional digestion at 35-37 ºC. Here, ozonation did effectively compensate for the slow hydrolysis of particulate substrate COD at low temperature (20 ºC) to overcome the rate-limiting step in anaerobic digestion, resulting in a better digester performance as compared to conventional digestion at 35-37 ºC.

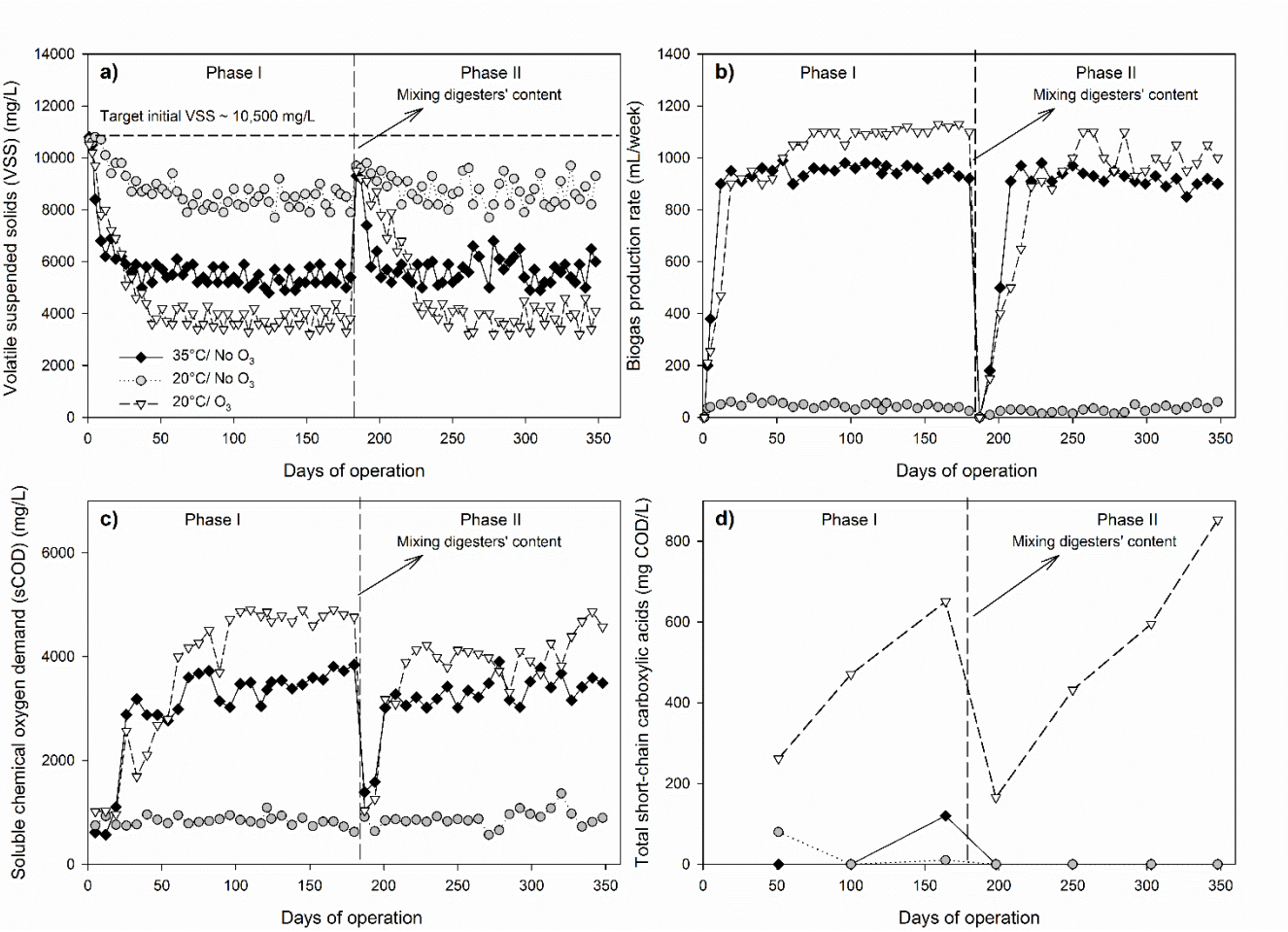

Fig 3.2. Performance of laboratory-scale anaerobic digesters under different operational conditions (low versus high temperatures; ozonated versus non-ozonated feed) showing a) VSS reduction b) Biogas production c) Soluble COD d) Total volatile fatty acids (VFA) concentrations. Phase I= Reactors were operated for 181 days based on operational conditions described in Table 1; Phase II= Contents of the three (3) reactors were mixed on Day 182, aliquoted back to each reactor and re-operated for 168 days based on conditions described in Table 3.1 to show reproducibility of phase I. The legend in panel a) also applies for b), c) and d); The average initial volatile suspended solid concentration of WAS was aproximately10,500 mg/L.

3.3.3 Soluble components

The residual of soluble COD (sCOD) concentrations were higher in the test reactor (20 °C/O_3) than in the positive (35 °C/ No O_3) and negative controls (20 °C/No O_3) reactors (Figure 3.2c).

This higher sCOD corresponded with a higher reduction in VSS and a lower $SMP_{VSSdestroyed}$ also observed with the test reactor (Table 3.3). The combined treatment of ozonation and anaerobic digestion of WAS at 20 ºC resulted in an average sCOD of 4,012 mg/L at steady state (Day 250-350), in contrast to anaerobic digestion of WAS alone at 35 ºC or 20 ºC, which produced an average sCOD of 3,390 mg/L and 1,059 mg/L, respectively (Table 3.3). On average, the initial influent sCOD fed to the reactors was 14,102 mg/L while the total effluent sCOD plus the amount of CH_4 in COD equivalent of the test reactor and the positive control reactor were 12,673 and 13,920 mg/L, respectively, showing mass balance of the sCOD component in the system. The mass balance closure was more than 90% for all the anaerobic digesters which seem a reasonable amount. The nature of the residual sCOD was investigated to determine how degradable it was. It was surprising to see that volatile fatty acids (VFAs) were also undetectable in the two control reactors. However, they accumulated in the test reactor (Figure 3.2d). The average total VFA concentrations in the test reactor were 460 and 580 mg COD/L in phases I and II, respectively, which correspond to 73% and 92% of the difference observed between the average sCOD concentrations in the test reactor and the positive control reactor. This suggests that methane production became the rate-limiting step in the test reactor while hydrolysis was the rate-limiting step in the positive control reactors.

Figure S2 shows each individual VFA concentration in the test reactor. Lactic acid and acetic acid were found to be the major components of the VFA profile for the test reactor. Previous reports have shown that in some natural, low temperature (< 20 ºC) environments and engineered systems, acetate tends to be a major methanogenic precursor (Akila & Chandra, 2007; Enright et al., 2009). It has been proposed that homoacetogenesis is an important biochemical pathway during low-temperature methanogenesis and that enhanced acetoclastic activity under conditions of low-temperature may arise from elevated autotrophic acetogenesis (Kotsyurbenko, 2005). Despite an increase in VFA level in the test reactor, its buffering capacity and a low organic loading maintained the pH around 7.49 (Table 3.3). This is in the optimal range of pH (6.5-7.6) to achieve maximal anaerobic digestion and biogas yield (Batstone & Jensen, 2011).

Table 3.3. Average performance of anaerobic digesters at steady-state during Phase I (Day 80-180) and Phase II (Day 250-350).

Reactor configuration	**20 °C/ O_3**			**35 °C/ No O_3**			**20 °C/ No O_3**		
	Phase I	Phase II	Average COD equivalent (mg/L)	Phase I	Phase II	Average COD equivalent (mg/L)	Phase I	Phase II	Average COD equivalent (mg/L)
pH	7.5±0.1	7.5±0.1	-	7.4±0.1	7.5±0.1	-	7.6±0.1	7.5±0.1	-
$sCOD_{out}$ (mg/L)	4,104±187	4,012±75	4,059±90[a]	3,475±130	3,390±95	3,432±72	839±48	1,059±48	949±30
Biogas volume (mL/week)	1,100±7	990±15	3,243±7 [a]	950±25	900±6	2,642±11	40±3	40±3	114±2
CH_4 (%)	61±1[b]	62±1	-	59±1	61±1	-	54±2	58±1	-
SMP_{VSSin} (mL CH_4/g VSS_{in})	242.5±4.8	221.8±9.6	-	202.6±13.3	198.4±14.2	-	7.8±1.6	8.4±1.1	-
$SMP_{VSSdestroyed}$ (mL CH_4/g $VSS_{destroyed}$)	390.7±6.2	361.4±4.6	-	441.5±3.9	469.2±8.6	-	51.3±2.5	69.6±4.1	-
VSS_{out} (mg/L)	3,748±26	3,817±73	5,371±35* [a]	5,348±46	5,703±66	7,846±36	8,379±53	8,692±69	12,120±39
VSS destruction (%)	62±4 [b]	60±2	-	46±2	43±2	-	16±2	13±5	-
Total COD_{in} (mg/L)			14,102±254			14,102±254			14,102±254
Total COD_{out} (mg/L) [% closure]			12,673±97[b] [90±2%]			13,920±82 [99±1%]			13,183±49 [93±1%]

Note: 35 °C/ No O_3= Digester operated at 35 °C fed with non-ozonated WAS; 20 °C/ No O_3= Digester operated at 20 °C fed with non-ozonated WAS; 20 °C/ O_3= Digester operated at 20 °C fed with ozonated WAS; sCOD= Soluble chemical oxygen demand; mL CH_4= Volume of methane; VSS= Volatile suspended solid; g VSS_{in}= Mass of volatile suspended solid into the digester; SMP_{VSSin} =Specific methane production using initial VSS; $VSS_{destroyed}$= Mass of volatile suspended solid destroyed in the digester; $SMP_{VSSdestroyed}$= Specific methane production using destroyed VSS; Total COD_{in}= Total initial COD into the reactors; Total COD_{out} = Total COD out of the reactors; Units: mg= Milligram; L= Litre; mL= Millilitre; g= Gram; %= Percent. * COD/ VSS ratio of 1.42 for WAS and COD/VSS ratio of 1.26 for anaerobic digestate, each mole of CH_4 contains 8 electron equivalents or 64 g of COD. Each mole of CH_4 has a volume of 22.4 L at STP (Rittmann & McCarty, 2001c); ±: standard error (number of replicates=3); [a] The standard error was measured by removing variability between the phases from the variability in the periods (n=6); [b] standard error was measured by error propagation.

3.3.4 Energy balance

Given that a better performance was achieved with the anaerobic digester operated at 20 ºC combining sludge ozonation than the conventional anaerobic digestion at 35 ºC, the energy requirements and gains for both systems were analyzed to evaluate their energy balance and sustainability. The full-scale digester is assumed to have a volume of 20,000 m^3 and to treat a WAS flow of 1,000 m^3/d at a total solids concentration of 3% (VSS/TSS ratio 0.7). Thus, the hydraulic and solids retention times are assumed to be 20 days and the organic loading rate to be 1.05 kg/m^3.d. Furthermore, the COD balances were assumed to be the same as the one observed experimentally, and the sludge and ambient temperatures were at 10 °C. Finally, all the electrical and thermal requirements were assumed to be from the biogas produced with a biogas to electricity efficiency of 35% and thermal heat efficiency of 70%.

For these conditions, at 35 °C without ozonation, the 43% reduction in VSS resulted in a biogas energy production of 226 GJ/d and a total energy loss for heating and mixing of 97 GJ/d. In contrast, operation of the anaerobic digester at 20 °C with sludge ozonation, which resulted in 60% VSS reduction, yielded a biogas energy production of 273 GJ/d and a total energy loss of 99 GJ/d (Figure 3.3). Combining these data, the net energy production from the 20 °C with sludge ozonation would be 35% higher than from the conventional anaerobic digestion at 35 ºC. This result is due to the higher degradability of the ozonated sludge since the heat energy inputs to raise the temperature from 20 °C to 35 °C were essentially replaced by equivalent amount of energy to deliver O_3 as pre-treatment. However, this observation suggests that from a digester performance perspective, suppling the energy as O_3 is more efficient than heating the digester at 35 °C.

The energy sustainability analysis showed that both operational scenarios (35 ºC without ozonation and 20 ºC with ozonation), had an *ESI* higher than 1, meaning that both systems are energetically sustainable. However, the 20 ºC-ozonated digester had a higher *ESI* of 2.88 than the conventional 35 ºC digester with an *ESI* of 2.33. This suggests that operating anaerobic digesters with municipal sludge at low temperature with solids ozonation should provide a more environmentally sustainable option than conventional anaerobic digestion at temperatures of 35 ºC or higher. Integrating ozone to low temperature anaerobic digestion seems to provide a more environmentally sustainable approach in cold environments, to overcome the physical, chemical and biological challenges associated with low temperature conditions, and enabling the harvest of more bioenergy. Such hybrid system may be attractive to countries with cold winter temperatures, where

heating anaerobic digesters to 35-37 ºC may imply a significant energy cost considering that the influent substrate is at a much lower temperature. Hence, operating a stand-alone anaerobic digester system at 35-37 ºC might not be an energetically sustainable solution. Application of a combined ozonation-low temperature digestion process may on the other hand, represent a better alternative.

According to Bolzonella et al. (2012), temperatures higher than ambient are required to maintain an efficient hydrolysis of substrates, but this entails a significant amount of energy to sustain the digestion process. Therefore, applications of low mesophilic temperatures (15-20 °C) have been studied. So far, good performances have been achieved for the anaerobic treatment of municipal wastewaters (Collins et al., 2005; Hill et al., 2001). Similar processes have been developed for the digestion of animal manure. Alvarez and Lidén (2009) used a mixture of llama, cow and sheep manure in an anaerobic digester operated at low temperature (18–25 °C) and observed methane yields between 70–140 mL CH_4/g VSS_{in}, as compared to 230 mL CH_4/g VSS_{in} at 35 °C. However, municipal wastewater and manure are much more degradable than WAS, which is highly limited by a slow hydrolysis rate. Consequently, anaerobic digestion of WAS at low mesophilic temperature requires excessively long SRT to achieve the same VSS reduction as high rate mesophilic digesters, making it impractical for economical application in the municipal sector. In the present study, it is shown that ozone pre-treatment of municipal sludge can effectively be used for low-temperature anaerobic digestion to lower sludge disposal, increase biogas yield, and importantly increase net energy production. This study suggests there is still great potential for expanding the application range of anaerobic digestion.

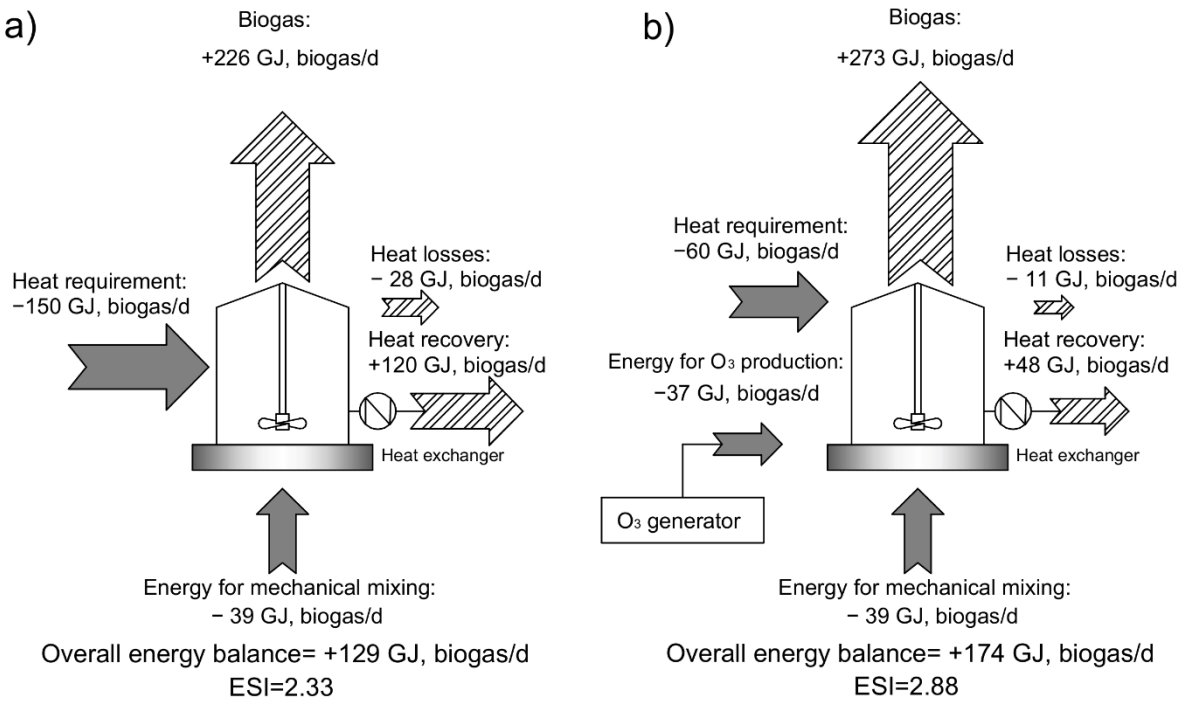

Figure 3.3. Comparison of energy budgets for different operational regimes a) Anaerobic digestion operated at 35 °C fed with raw WAS b) Anaerobic digestion operated at 20 °C fed with ozonated WAS (temperature of air, earth, and incoming sludge assumed to be 10 °C). *Note:* All energy units are reported in terms of biogas equivalent (GJ, biogas/d); ESI= Energy sustainability index; O_3= Ozone; GJ= Gigajoule; d= Day; += Energy gain; − = Energy expenditure.

3.4 CONCLUSIONS

The results of this research suggest that anaerobic digestion of ozonated municipal sludge is feasible at low mesophilic temperature (20 Cº), and even shows better performance than conventional anaerobic digestion at 35 ºC in terms of biosolids reduction and biogas yield per VSS loading rate at a 20 d SRT. Therefore, ozone can effectively substitute high supply of thermal energy, an approach which presents better sustainable features. Essentially, the energetic sustainability of this approach implies that economic and environmental benefits are to be gained from its implementation especially in environments with cold climate. Currently, there is a unanimous agreement on improving the sustainability of wastewater treatment systems. Towards this end, a further development and wider application of the hybrid technology described herein can enable better capture of bioenergy from biosolids treatment at a lower energy cost. A comprehensive, multidisciplinary research involving modeling will help implementing such

hybrid system at low temperature and at full-scale level. Such a study would highlight more practical issues regarding the application of this technology in view of rendering sludge handling at municipal wastewater treatment facilities more energy efficient and sustainable.

3.5 ACKNOWLEDGEMENT

The authors thank the operators of the wastewater treatment facilities for granting permission to collect samples. The contribution of Jean-François Lemay, Patrik Quessy and Marc-André St-Yves from the Centre National en Électrochimie et en Technologies Environnementales (CNETE) in Shawinigan, Quebec, Canada, is deeply acknowledged especially for their high-level discussion, and performing of the VFA analysis. The research was supported by grants from the Natural Sciences and Engineering Research Council (NSERC) of Canada, Air Liquid Canada and the Régie d'Assainissement des Eaux du Bassin LaPrairie. The project also benefited the support from the Mathematics of Information Technology and Complex Systems (MITACS) of Canada. The findings of this research work were presented at the ECI conference on Life Cycle Assessment and Other Assessment Tools for Waste Management and Resource Optimization, which was held in Cetraro (Calabria), Italy from 5-10 June 2016. The fruitful discussion therein is also acknowledged.

3.6 SUPPLEMENTARY INFORMATION

Table S3.1. Average characteristics of raw and ozonated waste activated sludge (WAS)

Parameter	TSS (mg/L)	VSS (mg/L)	VSS/TSS	NH_4 (N mg/L)	Total PO_4 (P-mg/L)
Raw WAS	12,441±27	9,882±34	0.79±0.01	16.2±1.3	202±12
Ozonated WAS	11,794±31	7,261±48	0.61±0.01	22.6±2.1	211±17

Note: WAS= Waste activated sludge; TSS= Total suspended solid, VSS= Volatile suspended solid; NH_4= Ammonium; PO_4= Phosphate; Units: mg= Milligram; L= Litre; mg/L= Concentration; N- mg: Milligram nitrogen; P- mg= Milligrams phosphorus; ±= Standard error (n= 3).

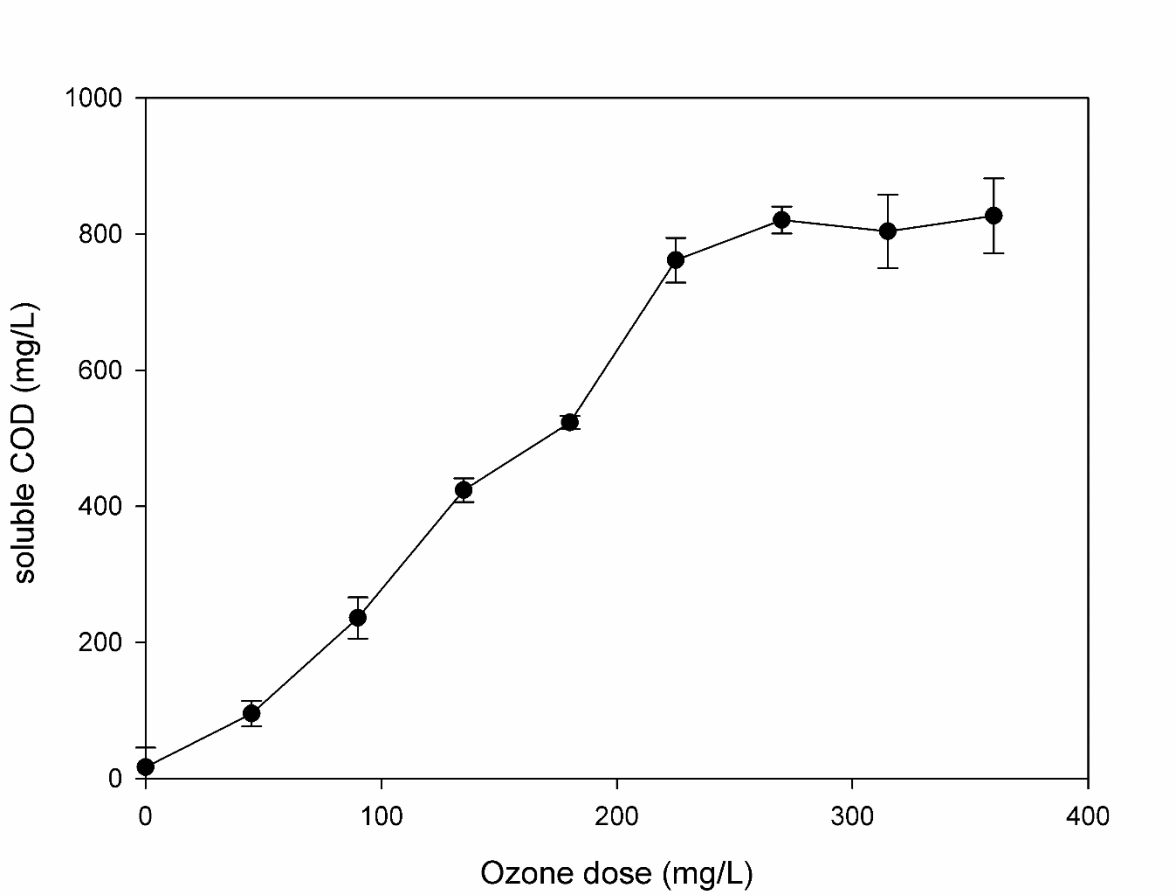

Figure S3.1. Solubilisation of waste activated sludge at different ozone doses; Each point on curve represents an average of 3 replicates (error bars indicate standard errors and VSS concentration was ~10,000 mg/L).

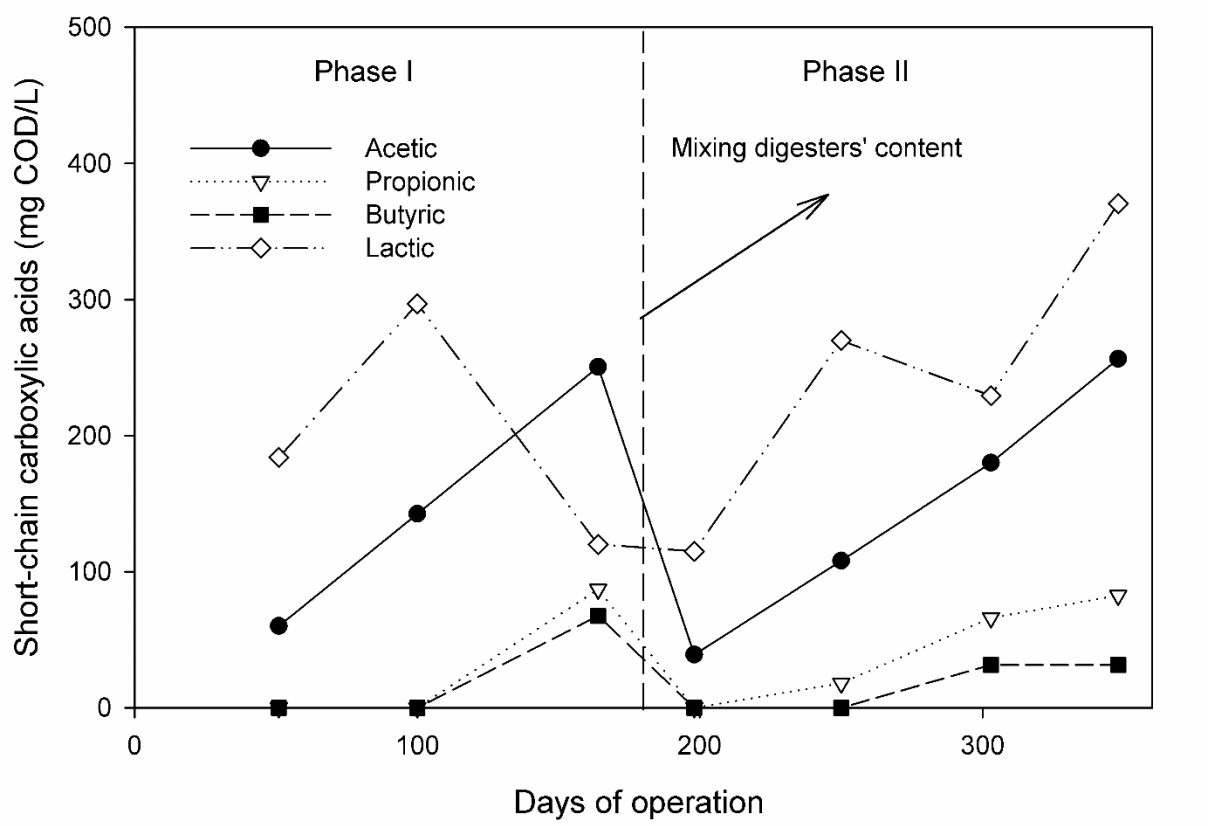

Figure S3.2. Concentrations of short-chain carboxylic acids in test reactor (20 °C/ O_3) over the experimental period.

Table S3.2. Paired-observation randomized complete block design (RCBD) ANOVA comparing average values of VSS, soluble COD and biogas production under the different treatments for the two experimental phases.

Comparison Treatment 1 – Treatment 2	*Average Treatment Effect*		*Average Phase Effect (Random Block)*		*Interaction Treatment×Phase*	
	Difference Treatment 1 – Treatment 2	*P* value	Difference Phase I – Phase 2	*P* value	Difference $(\text{Trt 1} - \text{Trt 2})_{\text{Phase I}} - (\text{Trt 1} - \text{Trt 2})_{\text{Phase II}}$	*P* value
VSS (mg/L)						
20 °C/ O_3–35 °C/ No O_3	1598±95	7.44×10^{-5}	209±62	0.028	289±135	0.099
20 °C/ O_3–20 °C/ No O_3	4631±104	1.52×10^{-6}	189±62	0.038	249±147	0.166
35 °C/ No O_3–20 °C/ No O_3	3033±19	8.41×10^{-9}	333±200	0.171	−40±26*	0.202
Soluble COD (mg/L)						
20 °C/ O_3–35 °C/ No O_3	625±252	0.068	−82±37	0.095	4±3	0.991
20 °C/ O_3–20 °C/ No O_3	3265±107	6.92×10^{-6}	71±130	0.614	−301±152	0.118
35 °C/ No O_3–20 °C/ No O_3	2640±159	7.77×10^{-5}	69±51	0.246	−305±225	0.247
Biogas production rate (mL/week)						
20 °C/ O_3–35 °C/ No O_3	152±15	4.98×10^{-4}	−78±19	0.014	−69±21	0.303
20 °C/ O_3–20 °C/ No O_3	1061±14	2.03×10^{-7}	−56.5±7	1.07×10^{-3}	−112±20	5.34×10^{-3}
35 °C/ No O_3–20 °C/ No O_3	909±20	1.24×10^{-6}	−22±12	0.146	−43±27	0.190

Note: Negative sign for treatment effect means Treatment 2 was higher than Treatment 1; negative sign for phase effect means Phase II was higher than Phase I; negative sign for Interaction means that difference between treatment was smaller during Phase II than during Phase I.

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CHAPTER 4

Effect of sludge ozonation on kinetics and biogas recovery in batch-fed and semi-continuous anaerobic digestion systems at low mesophilic temperature

Connecting text: Developing the findings of Chapter 3, we further explored the effect of ozone treatment of WAS or anaerobic digestate on the yield and rate of methane production during anaerobic digestion at 35 °C and 20 °C. In Chapter 4, analysis of methane production from anaerobic digestion of ozone-pretreated and untreated WAS or anaerobic digestate was carried out using a modified Gompertz model. The results provided insights on the kinetic parameters on WAS or digestate solubilization during ozone treatment. In the current chapter, also, the phylogenetic diversity of archaeal and bacterial populations of three bench-top anaerobic digesters at two different temperatures (35 °C and 20 °C) and feeding composition (ozonated biosolids vs non-ozonated biosolids) was studied. The main objective was to determine the effect of temperature and feeding composition on the microbial community structure of the digesters.

The results of this research is in preparation for submission to the following Journal:

Zeinab Bakhshi, Shameem Jauffur, Jean-François Lemay, Patrik Quessy and Dominic Frigon (In preparation, 2020), Effect of sludge ozonation on kinetics and biogas recovery in batch-fed and semi-continuous anaerobic digestion systems at low mesophilic temperature. *Journal of Bioresource Technology.*

4.1 INTRODUCTION

Anaerobic digestion of sewage sludge is a common process used in waste management to stabilize biosolids, reduce excess sludge production, generate useful energy and mitigate greenhouse gas emission (Kythreotou et al., 2014). Despite its proven potential and range of environmental benefits, further optimization of anaerobic digestion can increase the performance and contributions of the process to a wastewater treatment plant's sustainability (Appels et al., 2008; Nguyen et al., 2015). Some substrates such as sewage sludge result in low biogas yield due to the presence of a high concentration of recalcitrant volatile solids that negatively impact the methanogenenic COD transformation (Mata-Alvarez et al., 2014). Enzymatic hydrolysis of complex substrates has been reported to be the rate-limiting step during anaerobic digestion (Goswami et al., 2016; Zhang et al., 2014). Therefore, a number of strategies aimed at improving hydrolysis rates and solids degradability has been developed based on chemical, biological, mechanical, thermal and irradiation pre-treatment technologies (Carrere et al., 2010).

Previous studies have demonstrated the interest of ozonating waste activated sludge (WAS) before anaerobic digestion to oxidize refractory substrates and convert them into more biodegradable compounds (Meng et al., 2016; Weemaes et al., 2000). For conventional anaerobic digestion of sludge at 35-37 °C, Yeom et al. (2002) reported that an optimal ozone dose of 0.2 g-O_3/g-TSS enhanced biogas production. Chacana et al. (2017b) showed that ozonation initially and temporarily reduced biomass viability and activity, but enhanced methane production following a lag phase; their optimal ozone dose of 86 mg O_3/g COD increased the methane yield by up to 52%. Recently, the feasibility of anaerobic digestion at low mesophilic temperature has been demonstrated by operating benchtop anaerobic digesters, where anaerobic digestion of ozonated sludge at 20 °C led to a higher volatile suspended solids (VSS) destruction and methane production by 60% and 14%, respectively, compared to conventional anaerobic digestion at 35 °C (Bakhshi et al., 2018). Pre-treatment of WAS with ozone effectively overcame the rate-limiting step of hydrolysis to render the process of anaerobic digestion feasible at low mesophilic temperature (20 ºC). The results further showed a better biosolids reduction and biogas production for ozonated sludge digested at 20 ºC than for non-ozonated sludge at 35 ºC in conventional anaerobic digesters with a 20-day solids retention time (SRT). While pre-ozonation of WAS effectively enhanced anaerobic degradability, post-ozonation of recirculated digested sludge was found to be even more

efficient in increasing methane production during anaerobic digestion in continuously fed systems (Chapter5, 2020). Theoretical analyses have shown that this combined ozone-anaerobic digestion system operated at 20 °C is more energetically sustainable and can improve by 35% the net energy recovery over a typical anaerobic digester at 35 °C. Handling WAS through anaerobic digestion at ambient or lower temperature can thus hold economic incentives and avoid excessive heating costs especially in cold climate countries.

Although such hybrid system presents better performance and sustainable features, the extent of solids degradability induced by ozone and the kinetics of the anaerobic digestion process using ozonated substrates are unknown. This knowledge is important for the modeling, design and optimal operation of low mesophilic temperature anaerobic digestion systems. Kinetically, ozonation perform part of the disintegration and hydrolysis reaction of complex particle and macromolecules and may facilitate further enzymatic hydrolysis. By rendering certain compounds more degradable, ozonation can also increase the extent of substrate degradation. The current study aimed at investigating these effects using batch methane potential tests. The maximum methane production rate and ultimate methane yield in batch methane potential test have been analysed by different kinetic models such as the first-order, Monod, Contois and Andrews model (Kythreotou et al., 2014). One of the most successful model is the simplified generalized kinetics Gompertz model (Lee et al., 2017; Lo et al., 2010; Luo et al., 2012), which was used herein.

The current study expands on the results of a previous study (Bakhshi et al., 2018) with the objectives of providing some mechanistic explanations of the enhancement of methane production observed upon ozonation of WAS fed to anaerobic digesters operated at 20 °C and a 20-d SRT. The goal of the present study was to determine changes in the kinetics and yields of methane production for ozonated WAS or anaerobic digestate (AD) fed to single-stage anaerobic digesters operated under conventional conditions (35 °C) or low mesophilic temperature (20 °C). New biomass was raised to perform these experiments in batch biochemical methane potential (BMP) tests, and the results were compared with the previously published results for semi-continuous systems (Bakhshi et al., 2018). Since the anaerobic digestion process is driven by the synergistic effects of microorganisms (Mata-Alvarez et al., 2014), we present here new results on the methanogenic community structures under the various operating temperatures (20 °C and 35 °C) and feeding regimes (ozonated and non-ozonated WAS and AD) from previous semi-continuous

systems to understand the possible metabolic pathways associated with different anaerobic digestion operational conditions (Bakhshi et al., 2018).

4.2 MATERIALS AND METHODS

4.2.1 Inoculum and substrate

The inoculum was collected from a full-scale biomethanation plant in St-Hyacinthe (Quebec, Canada), that was treating 72,000 wet tons of WAS annually; it was used to seed four 7-L laboratory-scale anaerobic digesters. The inoculum had a total suspended solids (TSS) and volatile suspended solids (VSS) concentration of 23,150 and 16,190 mg/L, respectively. The digesters were operated for 150 days with a solids retention time (SRT) and a hydraulic residence time (HRT) of 20 days to grow enough acclimatized biomass for performing biochemical methane potential (BMP) assays. Two of the reactors were maintained at 20 °C while the other two were kept at 35 °C by means of a cooling/heating recirculating water-bath. Each digester was connected to a 2-L Tedlar® bag to collect the biogas. Fresh WAS to feed the lab-scale digesters was collected after the dissolved air-flotation thickener (DAF) from from the Régie d'Assainissement des Eaux du Bassin LaPrairie (RAEBL) Water Resource Recovery Facility (WRRF; Ste-Catherine (Quebec, Canada), which treats an average flow of approximately 65,000 m^3/d. The feed WAS was kept in the fridge until use and was added daily to the digesters; its TSS and VSS concentrations were 37,035 and 29,182 mg/L, respectively (Table S4.1).

4.2.2 Ozone treatment

Portions of the collected WAS from the full-scale plant, and anaerobic digestate (AD) from the laboratory-scale digesters were ozonated and used as feed in BMP assays. Ozone was generated using Ultra High Purity 4.3 oxygen (Praxair, Mississauga, Ontario) by an ozone generator (Ozomax, model OZO 3VTTL, Canton de Shefford, QC). The optimum ozone dose (mg/L) and the respective contact time with the sludge were determined based on chemical oxygen demand (COD) solubilization experiments (Bakhshi et al., 2018). Ozonation of the sludge was performed in a batch contactor vessel equipped with two inlet ports fitted with atomizer nozzles supplying ozone at a pre-determined rate. Ozonated WAS and AD samples were also collected during the operation of the ozone system to determine their characteristics (Table S4.1).

4.2.3 Batch system - Biochemical methane potential (BMP)

Methane yield was evaluated by performing BMP assays in 250-mL serological bottles at low (20 °C) and high (35 °C) mesophilic temperatures (Saha et al., 2011). Each bottle was filled with 105 g of well-mixed acclimated inoculum from the 7-L lab-scale anaerobic digesters operated at 20 °C and 35 °C, respectively. An amount of substrate was added to each bottle with an inoculum-substrate ratio (ISR) of 2:1 based on the VS. Previous studies have shown that an ISR that is too low (<0.5) can lead to acidification from volatile fatty acid (VFA) accumulation and can inhibit methane production (González-Fernández & García-Encina, 2009; Raposo et al., 2009). While the ISR was kept at 2:1, more inoculum and substrate were added to the BMP bottles incubated at 20 ºC (1.35 g of inoculum and 0.68 g of feed) than those maintained at 35 ºC (0.28 g of inoculum and 0.14 g of feed) to improve activity detection at the lower temperature. The tests were performed in quadruplicate and supplied with each of the following substrates: raw WAS, raw AD, ozonated WAS, and ozonated AD. Four bottles constituting the positive control were fed with starch at a concentration of 4,286 mg/L (Sigma-Aldrich, USA) and four additional bottles were used as blanks with only the inoculum and distilled water for volume adjustment. All BMP bottles were flushed with a mixture of 80% N_2 and 20% CO_2 for 3 minutes and sealed with rubber stoppers and aluminum crimps. One set of the BMP assay (24 serological bottles) was incubated at 20 ± 1 °C and another set with the same number of bottles and content was kept at 35 ± 1 °C in an incubator (New Brunswick™ Innova® 44, Germany) with constant shaking at 110 rpm for 35 days. Headspace biogas of the test and control BMP bottles was measured using a pressure gauge (F16, CECOMP Electronics, USA) and the methane content was analyzed at each sampling event by a gas chromatograph equipped with a thermal conductivity detector (GC-TCD) (CP 3800-GC, Varian, USA). The methane production (mL) during biogas sampling was determined using the Ideal Gas equation at standard temperature and pressure (STP) to determine the biogas volume and by multiplying the latter with the headspace methane content (%).

4.2.4 Semi-continuous system – Benchtop bottle digesters

Details on the operation of the bottles digesters have been published in Bakhshi et al. (2018); key elements are presented here to facilitate understanding by the reader. Three 1-L borosilicate glass bench-top digesters were operated in a semi-continuous mode for 350 days with an SRT and HRT of 20 days. Each bottle was inoculated with 17.6 g of anaerobic digestate and fed twice weekly

with fresh WAS (with a VSS concentration of 10,000 mg/L) collected from the sources described earlier. One of the bottle digesters was operated at 20 °C and fed with ozonated WAS (Test Digester). The sludge ozonation was performed as described in section 4.2.2. The second digester was maintained at 35 °C and fed with raw WAS (Positive Control) and the third digester was operated at 20 °C and fed with raw WAS (Negative Control). The bottle digesters were initially operated for 181 days to reach steady-state (Phase I). The contents of the three (3) digesters were thoroughly mixed on Day 182, re-dispensed in equal volume in each reactor and operated for an additional 168 days (Phase II) to show reproducibility of Phase I.

4.2.5 Analytical methods

Total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS) were analyzed according to Standards Methods (Rice et al., 2017). The pH of the digestate was monitored using a benchtop pH meter (Thermo Scientific Orion Star™ A211, USA). Ammonia nitrogen (NH_4^+-N) was determined based on the Berthelot method involving the conversion of NH_4^+ to monochloramine by hypochlorite and subsequent reaction with phenol at a pH of 13 (Rhine et al., 1998). The test was performed in microplates with the colorimetric measurements made using the SPECTRAmax® microplate spectrophotometer (Molecular Devices, CA, USA). The volume of biogas produced was measured using a pressure gauge (F16, CECOMP Electronics, IL, USA) and its methane content was analyzed by high-resolution gas chromatography equipped with a thermal conductivity detector (GC-TCD) (CP-3800 GC, Varian, USA).

4.2.6 Kinetic model evaluation

The modified Gompertz model (Eq. 4.1) was used to analyze the BMP methane production data as a function of time (Donoso-Bravo et al., 2010; Lee et al., 2017). The rate of anaerobic digestion was studied under different operational configurations using the modified Gompertz model (Lee et al., 2017; Wang et al., 2017).

$$Y_{CH_4} = P \exp\left(-\exp\left(\frac{r_m e}{P}(\lambda - t) + 1\right)\right) \qquad \text{Eq. 4.1}$$

where Y_{CH4} is the cumulative methane yield (mL CH_4/g VS_{in}), P is the ultimate methane yield (mL CH_4/g VS_{in}), r_m is the maximum methane production rate (mL CH_4/ g VS_{in}.d), e is the Euler's constant, λ is the lag period (day), and t is the digestion time (day).

Based on Eq. 4.1, it was assumed that the gas production rate is proportional to the microbial growth and metabolic activity (Nielfa et al., 2015). From this equation and best fitting procedures of the predicted and experimental methane yield data, r_m, P, and λ were determined using nonlinear optimization in MATLAB ver. R2011a using a least squares procedure and the Marquardt–Levenberg algorithm (Wang et al., 2017).

4.2.7 Microbial community analyses

A total of twelve (12) anaerobic digestate samples were collected for microbial community analyses from the three benchtop bottle digesters operated in semi-continuous mode. Samples were collected on Day 50 and 150 of Phase I, and Day 250 and 350 of Phase II. Total DNA was extracted from 0.25 g of wet micro-centrifuged pellets using the PowerSoil® DNA Isolation Kit (*MO BIO* Laboratories, Inc., Carlsbad, CA). Extracted DNA was used to amplify the bacterial and archaeal hypervariable region V_6-V_8 of the 16S rRNA gene using the forward fusion primer 926b_F and reverse primer 1392b_R (Engelbrektson et al., 2010). The primer sequences and PCR thermal cycles are provided in Table S4.2. Each 50 µL of PCR reaction mixture contained 0.5 µM of forward and reverse primer each, 5× Bioline PCR colorless buffer (Taunton, MA, USA), 2.75 mM $MgCl_2$, 250 µM dNTP (each), 12 ng/mL DNA template and 2.5 units Bioline Taq DNA Polymerase (Taunton, MA, USA) in UltraPure™ DNase/RNase-Free Distilled Water (Invitrogen, Carlsbad, USA). After a first purification of the resulting amplicons using the QIAGEN QIAquick PCR Purification Kit (USA), a second PCR was performed using barcoded primers (NEXTflex™ DNA Barcodes, Bioo Scientific) to enable multiplex sequencing and easy identification of samples. The PCR temperature profiles were 94 °C for 3 min, followed by 25 cycles of denaturation at 94 °C for 45 s, annealing at a 72.9 °C for 0.5 min and extension at 72 °C for 90 s before a final extension at 72 °C for 10 min. After a second purification, the amplicons were quantified using a PicoGreen assay (Quant-iT PicoGreen dsDNA Assay Kit, Thermo Fisher Scientific, USA). Purified and quantified libraries were combined in equimolar concentration and sequenced using the Illumina MiSeq 300 platform (Illumina, USA) at the McGill University and Genome Quebec Innovation Centre (Montreal, QC). Raw 16S rRNA tags in the FASTQ format were trimmed to remove primer sequences and merged using FLASh (Magoč & Salzberg, 2011). Demultiplexed sequences were denoised and chimera were removed with UCHIME (Edgar et al., 2011). The dataset was further filtered to remove singletons. High quality sequences were clustered

into operational taxonomic units (OTUs) with VSEARCH based on a sequence similarity of 97%, which is related to species level (Rognes et al., 2016). Taxonomic affiliation was performed using the Greengenes as reference database (Caporaso et al., 2010b). Alpha and beta diversities were determined using QIIME (Caporaso et al., 2010b), *BiodiversityR* package of the R-software (version 3.2.1), and Paleontological Statistics (PAST3) (Hammer et al., 2008b). Good's coverage, Chao1 richness and Shannon and Simpson diversity indices were calculated, and rarefaction curves produced. Similary, microbial populations assessed using the Bray–Curtis index were represented by principal coordinates analysis (PCoA). Canonical correspondence analysis (CCA) was used to analyze the microbial community data. CCA can detect relationships between environmental variables (in our experiment, temperature, VFA, sCOD, VSS, pH and ozone dose) and microbial community structure. All archaeal and bacterial communities with at least 2% relative abundance were taken for the CCA analysis. The CCA was performed using the PAST3 (Hammer et al., 2008b) software following its default settings. The length of an environmental parameter arrow in the ordination plot indicates the strength of the relationship of that parameter to community composition. Two-way analysis of variance (ANOVA) was performed in PAST3 (Hammer et al., 2008b) to evaluate any variation in the microbial community composition of the digesters.

4.3 RESULTS AND DISCUSSION

4.3.1 Methane yield in batch AD

The effect of ozonation on the cumulative methane yield was evaluated by BMP assays at 20 °C and 35 °C after 36 days of experimental run. At 20 °C, the ozonated WAS produced the highest methane yield (average of 100 mL CH_4/g VS_{in}) while the ozonated AD resulted in an average of 37 mL CH_4/g VS_{in} methane yield (Fig. 4.1). In the absence of ozone, the methane production did not exceed 48 mL CH_4/g VS_{in} for the WAS and 24 mL CH_4/g VS_{in} for the AD. For the reactors incubated at 35 °C, those fed with ozonated WAS yielded the highest methane production (average of 80 mL CH_4/g VS_{in}) compared to ozonated AD which led to an average of only 54 mL CH_4/g VS_{in}. The non-ozonated WAS and AD resulted in an average of 74 and 42 mL CH_4/g VS_{in}, respectively, while the controls produced an average of 73.2 and 34 mL CH_4/g VS_{in} for the starch fed and blank BMPs, respectively. Hence, ozonation led to a significant increase in methane production at both temperatures compared to non-ozonated substrates, and the methane potential of WAS was significantly higher than that of AD, as expected. The composition of the biogas was

not significantly impacted during ozonation as also reported in the literature (Chacana et al. (2017c). The CH_4 contents of the biogas from digestion were 51% ± 2% and 56% ± 5% for untreated and ozonated WAS, respectively, and they were 42% ± 2% and 45% ± 5% for untreated and ozonated AD, respectively.

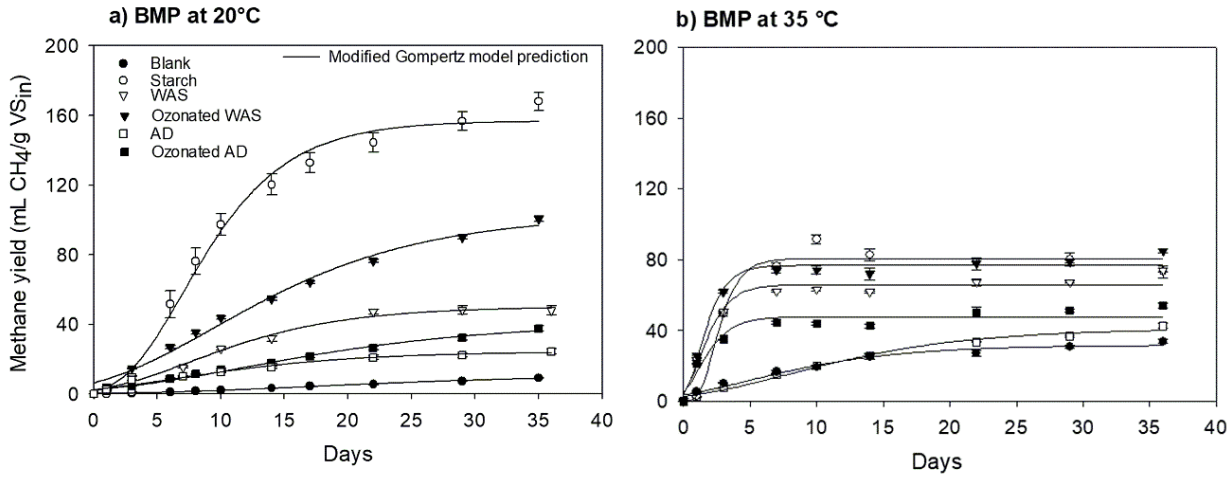

Fig 4.1. Methane yield from ozonated and non-ozonated substrates for batch-fed BMP assay at 20 °C (a) and 35 °C (b). Actual measured data are shown as marker dots and prediction by the modified Gompertz model is represented by the solid line. For the BMP assays, the samples were analyzed in quadruplicate (n=4). The data were not corrected based on the blank.

The BMP results reported in Fig. 4.1 can be described by several parameters: ultimate methane yields (P) seen as the plateaus, the maximum methane production rates (r_m) seen as the maximum slopes of the curves, and the lag times before significant methane production started (λ). To compare the effects of ozonation more precisely, these parameters were determined by fitting the cumulative methane production data (Fig. 4.1) with the modified Gompertz model (Eq. 4.1). For all BMP assays, the fits obtained between the experimental data and the model predictions were high ($R^2 > 0.93$, Table 4.1). Ozonation was also found to increase the lag time (λ) for the ozonated WAS than the untreated WAS at 20 °C by 34.1%; however, at same temperature, ozonating the digestate did not induce any significant change in λ. This was also the case for the assays at 35 °C where no significant change was found between ozonated substrates than non-ozonated ones. This is possibly due to the use of acclimatized biomass in each condition and the low lag times (<1 day) for all cases. The major impacts of ozone were on the ultimate methane yields and the maximum methane production rates; however, the importance of these effects appear to be

temperature dependent. Ozonation increased the ultimate methane yields much more at 20 °C than at 35 °C in both relative and absolute terms (Table 4.1). Conversely, ozonation increased maximum rates of methane production much more in absolute terms at 35 °C than at 20 °C, and increase that appears higher than that related to doubling of biological activity with each elevation in temperature by 10 °C (Rittmann & McCarty, 2001a).

Table 4.1 Kinetic parameters of modified Gompertz model for different substrates at 20 °C and 35°C in batch BMP assays.

	Ultimate methane yield (P) (mL CH_4/ g VS_{in})	Max. CH_4 rate (r_m) (mL CH_4/ g VS_{in}.d)	Lag period (λ) (day)	RMSE[a]	R^2
Digestion at 20°C					
Raw WAS	47.8±0.6[b]	4.0±0.2	0.9±0.2	4.54	0.99
Ozonated WAS	102.9±5.2	4.5±0.3	0.2±0.1	3.38	0.99
Raw digestate	24.0±1.7	1.1±0.7	0.0±1.4	0.99	0.99
Ozonated digestate	41.0±2.8	1.5±0.1	0.5±0.1	1.19	0.99
Controls					
Blank	11.3±1.0	0.3±0.0	4.3±0.6	0.23	0.99
Starch	157.0±5.3	12.6±1.5	2.4±0.7	7.09	0.99
Digestion at 35°C					
Raw WAS	65.9±2.0	19.6±4.3	0.1±0.3	3.91	0.97
Ozonated WAS	76.8±1.9	25.8±5.0	0.0±0.3	3.80	0.98
Raw digestate	41.1±1.9	1.9±0.2	0.1±0.9	1.62	0.99
Ozonated digestate	47.7±2.1	13.1±4.3	0.0±0.5	4.20	0.93
Controls					
Blank	31.6±1.7	1.8±0.3	0.0±1.2	1.87	0.97
Starch	80.6±2.4	28.2±9.2	1.2±0.5	7.33	0.93

[a] Root mean square of errors, [b] ±= Standard error (n= 4), The data are already corrected for the value of the blank control. Thus, reporting net activities.

Previous studies have mainly focused on determining the methane production from raw and ozonated substrates during anaerobic digestion at 35 °C. Lee et al. (2017) reported a methane yield of almost 80 mL CH_4/g VS_{in} after incubating ozonated WAS for 20 days, a quantity which is comparable to the level obtained in the present study. The difference in methane yield between ozonated and non-ozonated substrates may be due to a higher biodegradability induced by ozonation. Studies have shown that oxidative sludge pre-treatment solubilizes organic matter, thus increasing their accessibility to degrading microorganisms and improving anaerobic biodegradability (Carballa et al., 2007; Silvestre et al., 2015; Weemaes et al., 2000). Release of soluble components and increased biodegradability by ozonation may explain the higher methane yield from the ozonated substrates. On the other hand, anaerobic digestate has a low

biodegradability since anaerobic digestion already removed a portion of biodegradable matter leading to a lower methane yield as compared to WAS (Chacana et al., 2017c). To the best of our knowledge, no attempt has been made so far to anaerobically digest complex substrates (such as municipal wastewater treatment biosolids), at low mesophilic temperatures in a digester operated at a low SRT (here around 20 days). Some authors have studied the application of low mesophilic temperatures on animal manure. Chae et al. (2008) studied the effects of digestion temperature on the biogas yields and rates from the mesophilic anaerobic digestion of swine manure. A 17.4% reduction was observed when the digestion temperature was reduced from 35 °C to 25 °C. Temperature shocks from 35 to 25 °C led to a decrease in the biogas production rate.

4.3.2 Methane production in semi-continuous AD and microbial community structure analysis

Results on the community composition present in bottle digesters fed with ozonated and non-ozonated WAS in semi-continuous mode at 20 °C and 35 °C are reported here. Operation results have been reported previously (Bakhshi et al., 2018); key results are reported here to facilitate understanding by the reader. They are also compared with the batch BMP, which demonstrates the similarity in the results obtained by the two sets of experiments (Table 4.2).

Table 4.2. Methane yield in bottle reactors and BMP assays

	20 ºC with ozonated WAS		**35 °C with non-ozonated WAS**	
	Semi-continuous system	Batch system (BMP assay)	Semi-continuous system	Batch system (BMP assay)
Methane Yield (mL CH_4/gVSS_{in})	232.1 ± 7.2[a]	102.9±5.2[b]	200.5 ± 13.7[a]	65.9±2.0[b]

Note: [a] average methane yield for Phase I and Phase II; [b] ultimate methane yield. The standard error for the semi-continuous anaerobic digestion was based n= 6, and n= 4 for the batch mode anaerobic digestion.

The digesters fed with ozonated WAS and operated at a 20-day SRT and 20 °C produced an average cumulative methane yield of 232.1 ± 7.2 mL CH_4/g VSS_{in} (duplicate operation over time reported as Phase I and II), while those fed untreated WAS an operated at the same SRT but at 35 °C produced on average methane yield of 200.5 ± 13.7 mL CH_4/g VSS_{in} (Bakhshi et al., 2018). In the absence of ozone at 20 °C, the methane production was on average of 8.1 ± 1.3 mL CH_4/g VSS_{in}. These trends are in agreement with the findings of the BMP assays (Table 4.2), except that the ultimate methane yield in the BMP assay at 20 ºC with ozonated WAS and at 35 ºC with raw WAS were 44% and 35%, respectively lower than there counterpart in the semi-continuous digester operation. Similar results were also obtained by Holliger et al. (2017) where the ultimate methane yield from BMP assays were compared to biogas production from the same organic

materials in larger-scale anaerobic digester installations and suggested a correction factor of 0.8 for larger scale AD results.

Biochemical conversions in anaerobic digestion and the associated performance of the digesters are intimately linked to different groups of microorganisms. Thus, to gain further insights on the differences in the operation of the semi-continuous bottle digesters, the microbial community structures were studied using 16S rRNA gene amplicon sequencing. In total, 3,086,294 raw sequences were obtained from the 12 samples collected from the bottle reactors. After removing low quality sequences, a total of 1,326,460 high-quality reads were available for further downstream processing. The average number of sequences for *Bacteria* and *Archaea* were 109,954 and 23,645, respectively. At a 97% similarity threshold, the total number of operational taxonomic units (OTUs) generated was 3,895 and 216 for *Bacteria* and *Archaea*, respectively. Rarefaction analysis resulted in curves with high Good's coverage values (> 97%) indicating that the OTUs adequately estimated the microbial diversity of the reactors (Good, 1953) (Fig. S4.1). The diversity (Shannon and Simpson indexes) showed similar trends for the bacterial and archaeal populations with the 20 °C digesters fed non-ozonated WAS exhibiting lower diversity than the one fed ozonated WAS or the digesters operated at 35 °C fed non-ozonated WAS, which was typically due to a lower evenness for the communities in the digesters operated at 20 °C digesters fed non-ozonated WAS (Tables S4.3 and S4.4).

Marsland et al. (2019) studied the relationship of available energy fluxes with diversity, stability, and functional structure of microbial communities. The microbial context poses a strong mutual influence between the microbes and their chemical environment through the consumption and production of metabolites. In the present study, the higher diversity in the digesters operated at 35 °C with non-ozonated WAS and 20 °C with ozonated WAS than the 20 °C digesters fed with non-ozonated substrate was probably due to the energy fluxes during the production of metabolites. The higher the diversity shows more available biomass, and this results in more methane production. Sierocinski et al. (2018) investigated the link between methane production and diversity in laboratory anaerobic digesters by dilution and subsequent equilibration of biomass. The results showed a loss of the rarer species from communities and a positive relationship between methane production and the number of taxa, suggesting that rare species play an important role in methane-producing communities. Thus, any loss of diversity will likely reduce methane

production. Although temperature plays an important role in methane production, the enhanced availability of solubilized substrates induced by sludge ozonation, can effectively maintain bacterial and archaeal diversity and functional pathways to sustain conversion of substrates to methane.

Changes in bacterial and archaeal community structures were visualized by Principal Coordinate Analysis (PCoA) based on the Bray-Curtis distance metric (Fig.S4.3). The bacterial population of the three digesters was found to cluster distinctly, showing clear differences in composition. The close clustering of samples from the same digester shows similarity in phylogenetic structure than samples from the other digesters with different operational configurations. The archaeal community structure also changed over both time and between the three digesters. Archaeal communities of the 35 °C fed with non-ozonated WAS clustered together, indicating similar community composition. For the digester at 20 °C fed with ozonated WAS or with non-ozonated WAS, the community profiles shifted over time along the two axes of the plot from the initial consortium indicating distinctive patterns in the archaeal population structure.

The bacterial populations were dominated mainly by the phylum *Bacteroidetes*, followed in relative abundances by *Proteobacteria* and *Firmicutes* as subdominant phyla (Fig. S4.2a). The proportion of *Bacteroidetes* was higher at 20 °C, and even higher when the WAS feed was not ozonated leading to the lowest methanogenic production. This suggests that the *Bacteroides* appear to the primary hydrolysing phylum at lower temperature. Fourteen bacterial genera exhibited relative abundances ≥ 2% (Fig. 4.2a). Within the phylum *Bacteroidetes*, the genera *Prolixibacter*, and *Proteiniphilum* were the dominant genera, while the additional abundant *Bacteroidetes* populations at 20 °C with non-ozonated WAS feed were the family *Chitinophagaceae* and the genus *Bacteroides*. Within the *Firmicutes*, the genus *Sedimentibacter* was the most abundant (Fig. 4.2a). *Bacteroidetes* have been identified as a major phylum in solid-based digesters operated at 35 °C (Carballa et al., 2015). Well-known for their proteolytic activity, they have been reported to play an important role in protein degradation and fermentation of amino acids to acetate during anaerobic digestion (Chouari et al., 2005; Riviere et al., 2009b). The significant presence of *Proteobacteria* and *Firmicutes* in digesters treating municipal sludge has also been shown by other authors (Sundberg et al., 2013; Yang et al., 2014). *Prolixibacter*, *Proteiniphilum*, and *Sedimentibacter* genera have been shown to play a key role in fermenting sugars and producing

acetate and butyrate during anaerobic digestion (Chen & Dong, 2005; Nelson et al., 2011; Su et al., 2015).

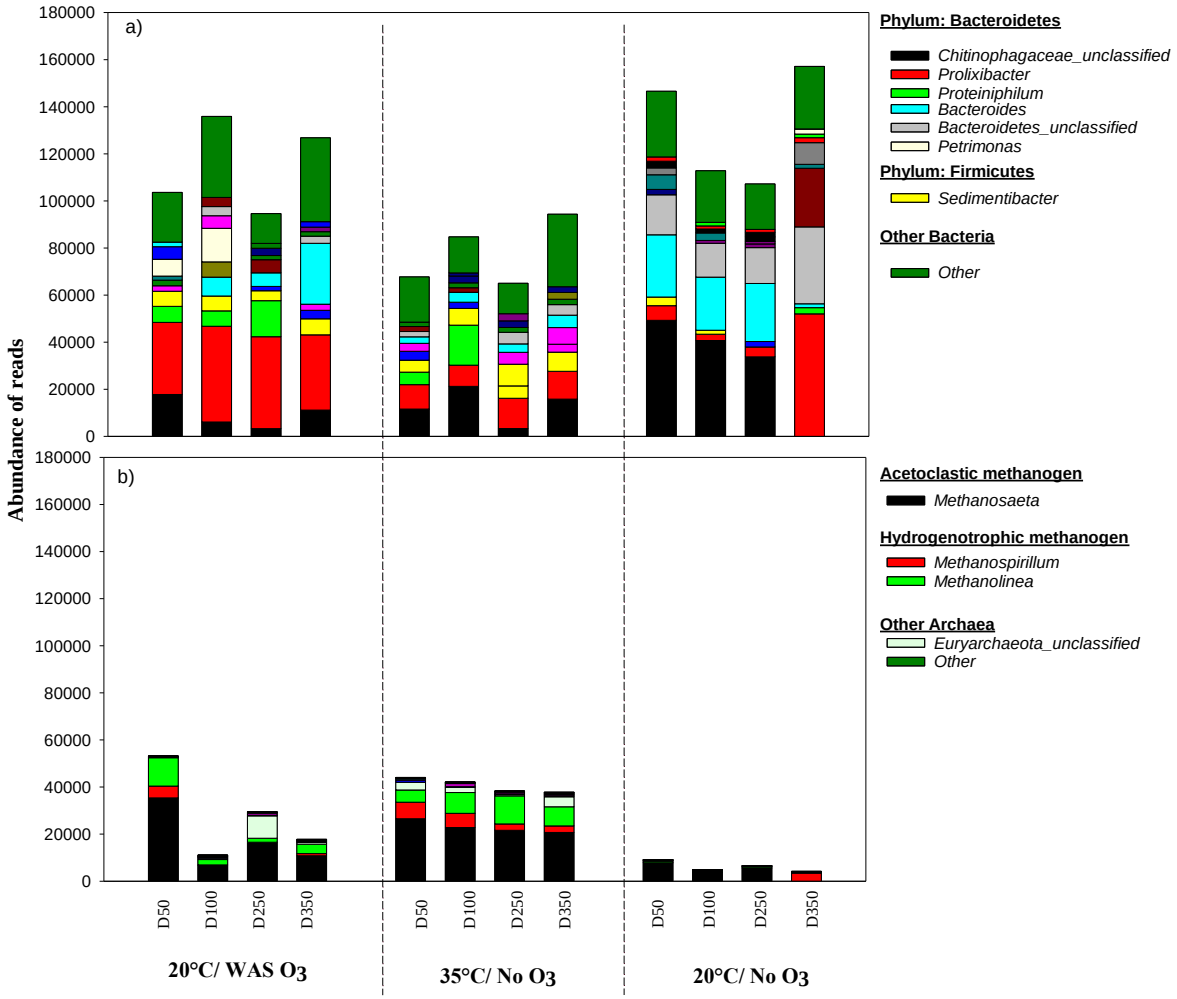

Fig. 4.2. Genus-level taxonomic classification of a) bacterial and b) archaeal populations in digesters operated at 20 °C receiving ozonated WAS (20 °C/ WAS O_3) or non-ozonated WAS (20 °C/ No O_3), or operated at 35 °C receiving non-ozonated WAS (35 °C/ No O_3). All digesters were operated at a 20-day SRT. Each phylum is coded with a unique color, and the height of each bar represents their abundance of reads. Phylogenetic groups accounting for ≤2% of all classified sequences are summarized in the artificial group "others".

The relative abundance of *Archaea* was higher in digesters operated at 35 °C receiving non-ozonated WAS and at 20 °C receiving ozonated WAS, while is was significantly much lower in digesters at 20 °C receiving non-ozonated WAS (p <0.05). The low methane production in the

latter digesters likely explains these data. *Euryarchaeota* dominated the archaeal populations in all three digesters (Fig S4.2b). At genus level, both acetoclastic (genus *Methanosaeta*) and hydrogenotrophic (genera *Methanolinea* and *Methanospirillum*) methanogens were present in digesters exhibiting substantial methane production (20 °C receiving ozonated WAS and 35 °C receiving non-ozonated WAS). The proportion of the *Methanospirillum* genus was significantly higher at 35 °C with non-ozonated WAS than at 20 °C with ozonated WAS (p <0.05). This genus has been described as a hydrogenotrophic methanogen capable of utilizing hydrogen, carbon dioxide and formate to produce methane (Kendall et al., 2007).

To determine which environmental gradients explained the variation in the observed phylogenetic structure, a constrained Canonical Correspondence Analysis (CCA) was performed using the 10 and 6 most abundant bacterial and archaeal genera, respectively (Fig. 4.3). Environmental variables (arrows) and genera (open circles) projecting at the same angle show high positive (i.e., same direction) or negative (i.e., opposite direction) correlations. Thus, on the one hand, ordination on the bacterial community showed that ozonation of substrate and VFA concentration (which were higher in digesters operated at 20 °C receiving ozonated WAS) correlated positively with the first canonical axis (CCA1, accounting for 68.3% of the variance in bacterial genera distribution). On the other hand, temperature was more correlated with axis 2 (CCA 2, accounting for 19.9% of the variance in the bacterial genera). As a potential explanatory variable, pH did not show a strong relationship the bacterial populations projected in this 2D plane because of the short length of the arrow line, which may be due to the measured pH generally remaining close to neutrality in all three digesters.

Town et al. (2014), observed the proliferation of selected organisms within the bacterial community with a strong positive correlation to reactor performance measures including methane and acetate production. In the current experiment, the abundance of *Prolixibacter* (of the phylum *Bacteroidetes*) was strongly correlated to substrate ozonation and VFA accumulation, while the genus *Bacteroides* and other unclassified OTUs within the phylum *Bacteroidetes* were negatively correlated to these variables but positively correlated to the digestate VSS (Fig. 4.3a). García-Ruíz et al. (2020), also reported a correlation between *Bacteroidetes* and digestate volatile solids concentrations. These data suggest a variety of functions and ecological niches within the phylum *Bacteroidetes*.

The abundance of *Sedimentibacter* and *Clostridium* (both of the phylum *Firmicutes*) appeared influenced positively by higher temperatures and sCOD concentrations. As indicated, ozone and temperature were strongly linked to the bacterial community based on their vector length.

Studies have shown the influence of environmental parameters on the microbial community structure, mainly focusing on the methanogenesis pathway due to its importance in generating CH_4 as a renewable energy source and reducing toxic compounds (García-Ruíz et al., 2020; Karakashev et al., 2005b). Thus, operational parameters such as sCOD, temperature, VSS and VFA can change the methanogenic community structure and affect the performance of the digestion process. Furthermore, previous studies demonstrated that the diversity in microbial community composition in mesophilic digestion was higher than thermophilic digestion (Bassani et al., 2015), and therefore the functional redundancy in mesophilic conditions is higher compared to the thermophilic ones. Thus, temperature might influence the diversity of the microbial community in the 35 and 20 °C digesters. Ordination of the archaeal communities showed a positive correlation of the *Methanolinea* genera with sCOD level and temperature while the *Methanosaeta* genera was influenced by the VSS concentration. Observations of the behaviours of *Methanosaeta* in these reactors suggest that acetoclastic methanogenesis is the first to appear at low methane fluxes, which are restricted by temperature or hydrolysis. As discussed previously the microbial context poses a strong mutual influence between the microbes and their chemical environment through energy fluxes and consumption and production of metabolites (Marsland et al., 2019). In the present study, the higher abundance of *Methanosaeta* in the digesters operated at 35 °C with non-ozonated WAS compared to 20 °C with ozonated WAS and 20 °C digesters fed with non-ozonated substrate was probably due to the energy fluxes during the production of the metabolites.

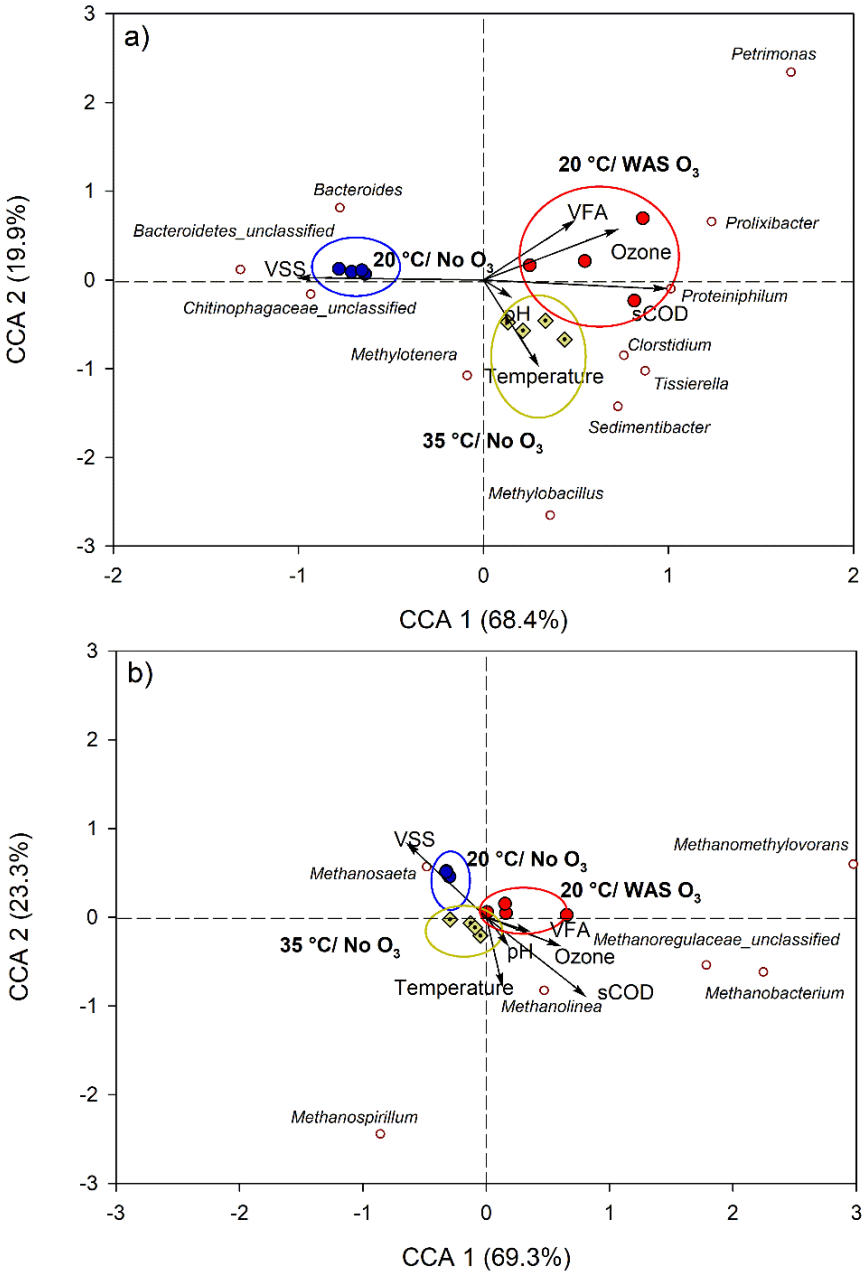

Fig. 4.3. Canonical correspondence analysis (CCA) showing correlations between a) Bacterial and b) Archaeal communities with environmental variables (digestate VSS, temperature, ozone, pH, soluble COD and VFA concentration). The process variables are represented by black arrows and the bottle digesters by different colored markers. Key bacterial and archaeal genera are shown as open circles.

Although temperature has a strong impact on the development of microbial communities (De Vrieze et al., 2015; Labatut et al., 2014), the type of feed supplied to the digester also influences microbiomes of anaerobic digesters (Zhang et al., 2014). In the present case, ozonation of the substrate maintained the population of key genera involved in methanogenesis to sustain methane production even at low mesophilic temperature. These results indicated that operational conditions such as temperature and substrate (ozonation) play an important role in shaping the microbial community in anaerobic digesters, supporting the findings of previous studies (Amani et al., 2010; Sundberg et al., 2013).

4.4 CONCLUSIONS

The effects of sludge ozonation on the performance of batch and semi-continuously operated anaerobic digesters were evaluated by monitoring the methane production and VSS concentration at low (20 ºC) and high (35 ºC) temperature. A higher methane yield was obtained at both temperatures for the batch system with ozonated substrates compared to non-ozonated feed. Similar findings were found with the semi-continuously fed bottle digesters where pre-treatment of the WAS effectively improved the performance of the digestion process at low mesophilic temperature (20 ºC) without the requirement of additional heat energy. Ozonation led to a significant increase in methane production at both temperatures compared to the non-ozonated feed, and the methane potential from anaerobic digestion of WAS was significantly higher than that of anaerobic digestate. Ozonation increased the ultimate methane yields much more at 20 °C than at 35 °C in both relative and absolute terms. Conversely, ozonation increased maximum rates of methane production much more in absolute terms at 35 °C than at 20 °C. rRNA gene amplicon sequencing analyses revealed distinct bacterial and archaeal population structures and composition between digesters fed with ozonated and non-ozonated substrates at both temperature regimes. These findings show the importance of temperature and feed type in shaping the microbial diversity and community structure, which are closely linked to functional stability and performance of the digesters.

4.5 ACKNOWLEDGEMENTS

The authors thank the operators of the wastewater treatment facilities for granting permission to collect samples. The contribution of Antonio Avalos Ramirez and Marc-André St-Yves from the

Centre National en Électrochimie et en Technologies Environnementales (CNETE) in Shawinigan, Quebec, Canada, is deeply acknowledged especially for their high-level discussion. The research was supported by grants from the Natural Sciences and Engineering Research Council (NSERC CRDPJ 5008) of Canada, Air Liquid Canada and the Régie d'Assainissement des Eaux du Bassin LaPrairie. The project also benefited the support from the Mathematics of Information Technology and Complex Systems (MITACS) of Canada.

4.6 SUPPLEMENTARY INFORMATION

Table S4.1. Average characteristics of raw and ozonated waste activated sludge (WAS) and anaerobic digestate (AD)

Parameter	TSS (mg/L)	VSS (mg/L)	VSS/TSS	sCOD (mg/L)
Raw WAS	37,035±927	29,182±934	0.79±0.06	122.6±9.1
Ozonated WAS	34,994±1031	25,671±448	0.73±0.02	1,446.2±104
Raw AD	23,150±827	16,190±749	0.73±0.01	1,052±109
Ozonated AD	19,500±906	14,800±563	0.76±0.02	3,623±214

Note: Fresh WAS was collected from the LaPrairie full-scale wastewater treatment plant. WAS= Waste activated sludge; AD=Anaerobic digestate; TSS= Total suspended solid, VSS= Volatile suspended solid; sCOD= soluble chemical oxygen demand; Units: mg= Milligram; L= Litre; mg/L= Concentration; ±= Standard error (n= 3).

Table S4.2. Primer sequences and PCR thermal cycles of PCR 1 and PCR2.

Primers		Primer Sequence (5'CS--3'CS)	Optimized PCR thermocycling programs
PCR1 (Engelbrektson et al., 2010)	926b_F	AAA CTY AAA KGA ATT GRC GG	Initial denaturation at 91°C for 3 min; then 25 cycles of: 95°C for 30 s, 62.2°C for 45 s, and 72°C for 90 s; then final extension at 72°C for 10 min.
	1392b_R	ACG GGC GGT GTG TRC	
PCR2	Uniprimer1	AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCC CTA CAC GAC	Initial denaturation at 94°C for 3 min; then 25 cycles of: 94°C for 45 s, 72.9°C for 30 s, and 72°C for 90 s; then final extension at 72°C for 10 min.
	Uniprimer2	CAA GCA GAA GAC GGC ATA CGA GAT CGA TGT GTG ACT GGA GTT C	

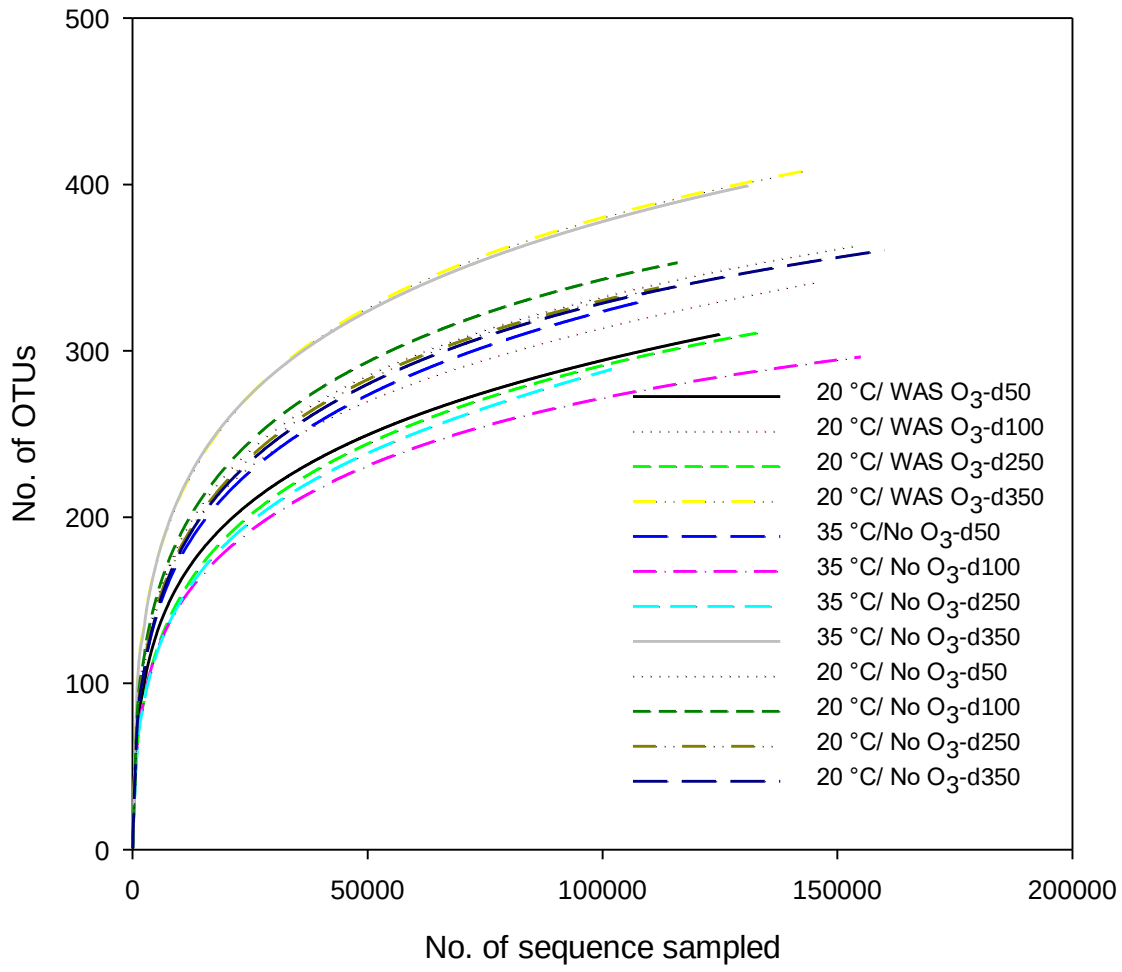

Fig. S4.1. Rarefaction curves of bacterial and archaeal OTUs defined by 3% sequence variation in anaerobic digestate samples for 20 °C/ WAS O_3, 20 °C/ No O_3 and 35 °C/ No O_3 collected on different days. *Note:* 20 ºC-O_3 = digester fed with ozonated WAS and operated at 20 ºC, 35 ºC/NoO3 = digester fed with raw WAS and operated at 35 ºC, and 20 ºC-NoO_3 = digester fed with raw WAS and operated at 20 ºC; OTU = Operational taxonomic unit; D = denotes the sampling day.

Table S4.3 Richness and diversity estimators of bacterial communities in semi-continuously fed anaerobic digestion systems (α = 0.03).

Reactor	Days of operation	Diversity indices				
		No. of OTU (*S*)	Shannon (*H*)	Simpson (*1-D*)	Evenness *H*/ln(*S*)	Chao-1
20 °C/ WAS O_3	50	283	2.794	0.8645	0.4949	324.8
	150	324	3.003	0.8813	0.5195	442.8
	250	294	2.402	0.7903	0.4227	353.8
	350	388	3.104	0.8799	0.5208	451.1
35 °C/ No O_3	50	312	3.249	0.9226	0.5657	387.1
	150	291	2.777	0.8715	0.4896	374.3
	250	271	2.931	0.9095	0.5232	402.7
	350	379	3.472	0.9321	0.5848	464.4
20 °C/ No O_3	50	347	2.685	0.8347	0.459	435.1
	150	338	2.57	0.8103	0.4413	386.4
	250	323	2.562	0.8238	0.4435	372.2
	350	345	2.49	0.8168	0.4261	410

Note: 20 ºC/ WAS O_3 = digester fed with ozonated WAS at 20 ºC, 35 ºC/ No O_3 = digester fed with raw WAS at 35 ºC, and 20 ºC/ No O_3 = digester fed with raw WAS at 20 ºC; OTU = Operational taxonomic unit.

Table S4.4 Richness and diversity estimators of archaeal communities in semi-continuously fed anaerobic digestion systems (α = 0.03).

Reactor	Days of operation	Diversity indices				
		No. of OTU (*S*)	Shannon (*H*)	Simpson (*1-D*)	Evenness *H*/ln(*S*)	Chao-1
20 °C/ WAS O_3	50	21	1.359	0.636	0.4462	21
	150	18	1.308	0.5867	0.4525	18.33
	250	18	1.395	0.7017	0.4827	18
	350	21	1.248	0.5728	0.41	22
35 °C/ No O_3	50	20	1.263	0.5922	0.4214	21
	150	20	1.34	0.64	0.4473	20.5
	250	19	1.126	0.5789	0.3825	19
	350	14	0.9124	0.497	0.3457	15
20 °C/ No O_3	50	17	0.6944	0.2796	0.2451	17
	150	16	0.8196	0.3327	0.2956	16
	250	16	0.7154	0.2848	0.258	17
	350	16	0.682	0.2626	0.246	16.5

Note: 20 ºC/ WAS O_3 = digester fed with ozonated WAS at 20 ºC, 35 ºC/No O_3 = digester fed with raw WAS at 35 ºC, and 20 ºC/ No O_3 = digester fed with raw WAS at 20 ºC; OTU = Operational taxonomic unit.

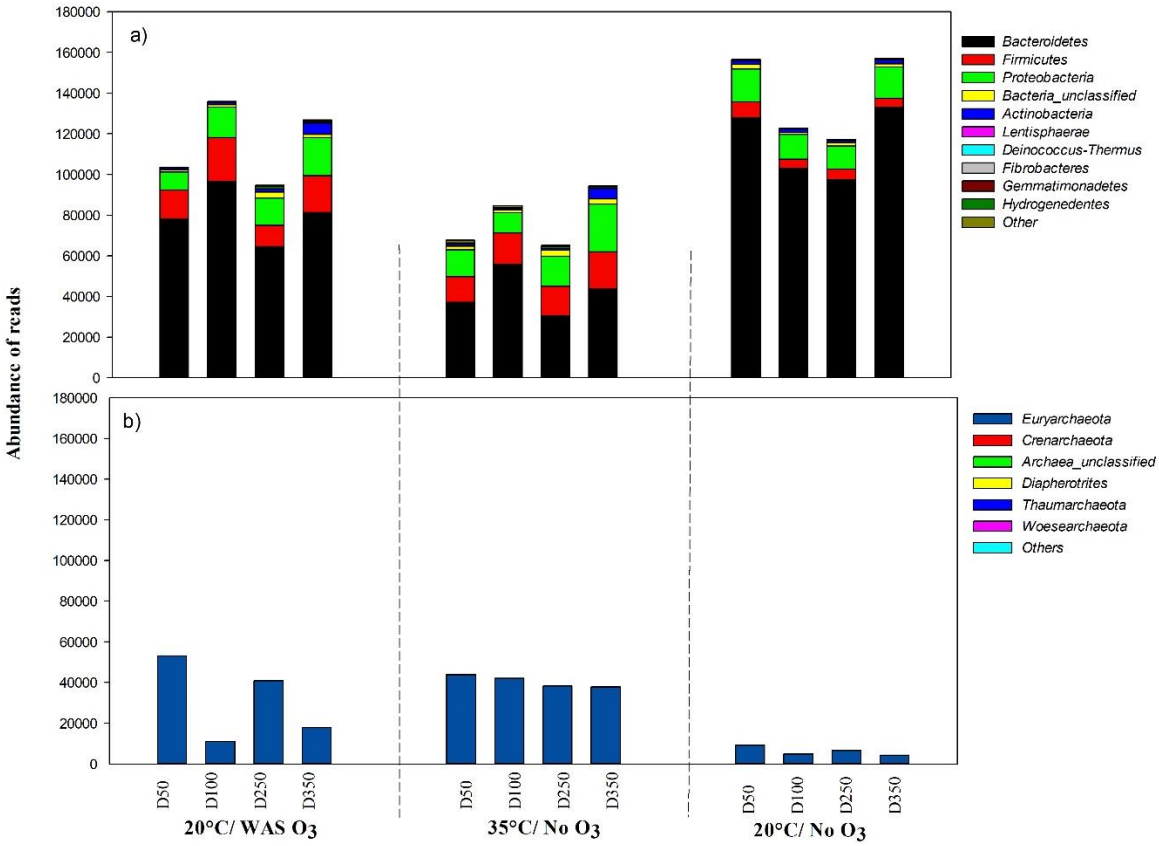

Fig. S4.2 Phylum level taxonomic classification of a) bacterial and b) archaeal populations in digesters operated at 20 °C receiving ozonated WAS (20 °C/ WAS O_3) or non-ozonated WAS (20 °C/ No O_3), or operated at 35 °C receiving non-ozonated WAS (35 °C/ No O_3). All digesters were operated at a 20-day SRT. Each phylum is coded with a unique color, and the height of each bar represents their abundance of reads. Phylogenetic groups accounting for ≤2% of all classified sequences are summarized in the artificial group "others".

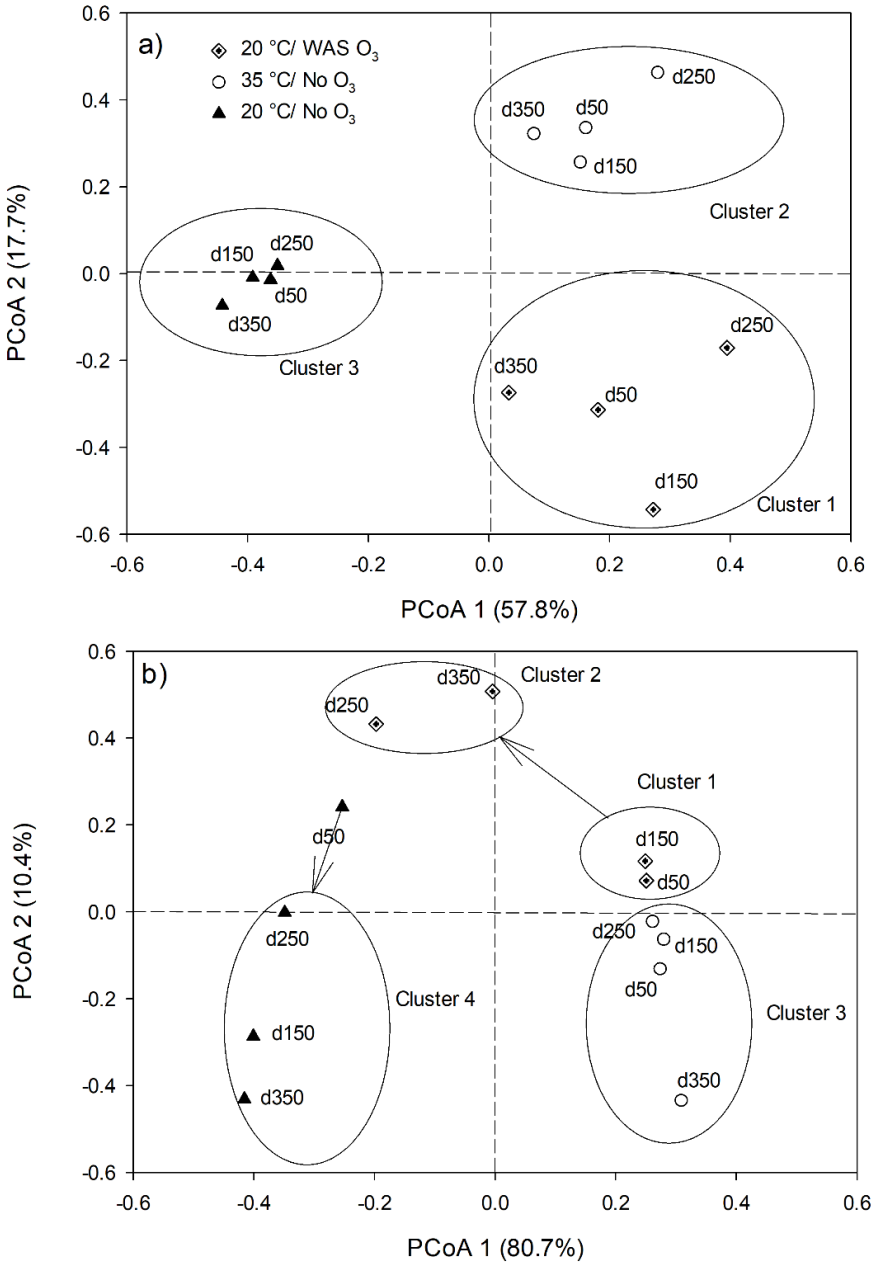

Fig. S4.3. Principal coordinate analysis (PCoA) of a) Bacterial and b) Archaeal communities of the bottle digesters. Ordination of samples was based on the Bray-Curtis distance metric at 3% cutoff level. Markers of the same type denote samples from the same reactors but at different sampling days. The circles show possible clustering of samples based on similar phylogenetic composition.

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CHAPTER 5

Maximization of Energy Recovery and Reduction of Biosolids Production by Combining Ozonation Treatment and Anaerobic Digestion at Low Mesophilic Temperature

Connecting text: Building on the findings of Chapter 3 and 4, Chapter 5 further examined the feasibility of anaerobic digestion of ozonated biosolids at low mesophilic temperature by answering the following research questions: a) Does ozone treatment of WAS, or recirculated anaerobic digestate, affect sludge reduction and biogas production? b) What is the optimum SRT and point of ozonation during the anaerobic digestion process? c) Does decoupling of SRT and hydraulic retention time (HRT) have an impact on biogas production and sludge reduction? d) What are the impacts of temperature (35 ºC vs. 20 ºC), ozonation (ozonated vs. non-ozonated substrates), and SRT (coupled and decoupled SRT from HRT) on the composition of the archaeal and bacterial populations and the community structure of the anaerobic digesters? To answer these questions, four lab-scale anaerobic digesters were operated: (i) at 20 °C, fed with ozonated WAS, (ii) at 20 °C, fed with ozonated anaerobic digestate, (iii) 35 °C, fed with raw WAS, and (iv) 35 °C, fed with ozonated WAS. These experimental results address gaps on how the microbial communities change during anaerobic digestion at different temperatures and feeding compositions, and how this change relates to their effectiveness for enhanced CH_4 production.

The results of this research will be submitted to the journal of *Environmental Science & Technology* by the end of 2020, under the title "Maximization of Energy Recovery and Reduction of Biosolids Production by Combining Ozonation Treatment and Anaerobic Digestion at Low Mesophilic Temperature". The authors will be listed as Zeinab Bakhshi, Shameem Jauffur, and Dominic Frigon.

5.1 INTRODUCTION

The treatment of an increasing volume of domestic and industrial wastewater by existing or newly commissioned water resource recovery facilities (WRRFs) involving biological processes results in large quantities of waste biosolids (Wang et al., 2008). They are unavoidable by-products of wastewater treatment processes and are generated at rates of between 70–90 g/person equivalent per day, or 1 dry ton/10,000-person equivalents per day (Fytili & Zabaniotou, 2008). Furthermore, biosolids handling and disposal can account for approximately 50% of the total operational costs of wastewater treatment facilities (Kroiss, 2004). Therefore, wastewater utilities are actively seeking to implement more sustainable treatment and disposal methods.

Anaerobic digestion is one of the optimal treatment technologies for handling waste activated sludge (WAS) because it reduces disposal of biosolids and produces renewable energy in the form of biogas. COD transformation in the digestion process commences with the slow reactions of disintegration and hydrolysis of insoluble compounds, followed by their fermentation to organic acids and hydrogen, and finally, methane (CH_4) synthesis from these last products (Lyberatos & Skiadas, 1999). Due to the rate-limiting step of disintegration and hydrolysis, conventional anaerobic digesters are operated at 35-37 °C to maintain a high hydrolysis rate and to allow for a digester design with a relatively low solid retention time (SRT) of around 20 days (Rittmann & McCarty, 2001a).

In chapter 3, we demonstrated that operating high-rate (~20-day SRT) anaerobic digesters at a low mesophilic temperature (20 °C) was feasible by combining it with sludge ozonation, and that this digester configuration enhanced both volatile suspended solids (VSS) destruction and methane yield over a conventional 35 °C process . Ozone is a powerful oxidant capable of oxidizing a wide range of organic and inorganic compounds (Chu et al., 2008). Pre-treatment of WAS by ozone disintegrates and partly solubilizes particles, inactivates biomass (including pathogens), and transforms non-biodegradable particulate organic matter into biodegradable substrates (Chacana et al., 2017a; Isazadeh et al., 2014). The hydrolysis and disintegration of particulate matter by ozonation remove the rate limitation imposed on the digester performance at 20 °C, while transformation of non-biodegradable organics to degradable substrates enhances solids destruction and their conversion to methane. Consequently, the input of energy to increase the temperature to

35 °C can effectively be replaced by WAS ozonation without major changes to the conventional process (Bakhshi et al., 2018).

The proposed new process (at laboratory scale), with operating anaerobic digesters at low mesophilic temperature (20 °C) while ozonating the WAS, achieved 37% higher VSS destructions than conventional anaerobic digestion at 35 °C and an enhanced biogas production by 14% (Bakhshi et al., 2018). However, it is not clear that ozonating the WAS is the ideal point of treatment to maximize enhancement by ozone. Would applying ozonation to the anaerobic digestate provide better results than applying it to the WAS because it would focus the oxidative action of ozone on the least degradable solids fraction? Furthermore, would decoupling the hydraulic retention time (HRT) from the SRT further enhance the reduction of biosolids and production of biogas from ozonated WAS or digestate? And finally, what are the impacts of temperature (35 ºC vs. 20 ºC) and ozonation (ozonated vs. non-ozonated substrates) on the composition of the archaeal and bacterial populations and the community structure of the anaerobic digesters? These questions are crucial to optimize and further develop the new process.

The microbial composition drives the digestion process. Therefore, it is important to investigate the impact of temperature (35 ºC vs. 20 ºC) and ozonation on the bacterial and archaeal populations. Understanding the microbial community structure would provide important insights on the underlying mechanisms, promoting methane production in such a hybrid ozonation-anaerobic digestion system, considering that microbial community dynamics influence functional stability and respond to process disturbances (De Vrieze et al., 2013).

The objectives of this study were, therefore, to provide answers to these key technical questions in view of optimizing the system in the future, and eventually, easing its implementation on a larger scale. This was achieved by operating benchtop anaerobic sequencing batch reactors (ASBRs) under different operational conditions of temperature (20 ºC vs 35 ºC) and SRTs. Finally, microbial community structures were studied using high throughput 16S rRNA gene amplicon sequencing to determine any variation incurred by the different operational conditions.

5.2 MATERIALS AND METHODS

5.2.1 Reactor operation

Four Plexiglas laboratory-scale anaerobic sequencing batch reactors (ASBRs), with a total and working volume of 4 and 2 L, respectively, were operated in parallel for 385 days (Fig. 5.1). The content of the reactors was agitated by means of overhead mixers (Model 1750, Arrow Engineering Mixing Products, USA). The temperature of the reactors (either 20 °C or 35 °C) was maintained by means of a recirculating water bath (IsoTherm model 250LC, Fisher Scientific, MA). The temperature and pH of the reactors were monitored using the Apex AquaController (model APEXLSYS, Neptune Systems, San Jose, CA). Each reactor was connected to a 2-L Tedlar® bag to collect biogas. For the reactor start-up, anaerobic sludge from a full-scale digester, located in St-Hyacinthe (Quebec, Canada), was used to inoculate the digesters. The digesters were fed with fresh WAS (~30,000 mgVSS/L) collected bi-weekly from the Régie d'Assainissement des Eaux du Bassin LaPrairie (RAEBL) WRRF (Quebec, Canada). Two ASBRs were operated at 35 °C: a control fed with raw WAS (35 °C/ No O_3), and a test reactor fed with ozonated WAS (35 °C/ WAS O_3); two other ASBRs were operated at 20 °C: one fed with ozonated WAS (20 °C/WAS O_3), and one fed with raw WAS and recirculated ozonated digestate (20 °C/AD O_3) (Fig. 5.1). To test the impact of SRT and HRT/SRT decoupling on the digester performance, the 385-day operation was segmented in distinct periods of 55-70 days. During the first period, considered a start-up period, the SRTs of the digesters were set at 20 days. Then, the SRTs in subsequent consecutive periods were adjusted to 15, 10, and 20 days, respectively. Finally, the SRT was increased above the HRT (i.e., the SRT and HRT were decoupled) to concentrate and recirculate part of the digestate, to maintain the same level of solids in the three ozonated digesters as observed in the control (35 °C/ No O_3). The organic loading rate for WAS varied from 3g VSS/d to 6 gVSS/d due to the change in SRT from 20 to 10 days. Recirculation of AD was performed with a volume of AD to the volume of WAS ratio of 1/2.

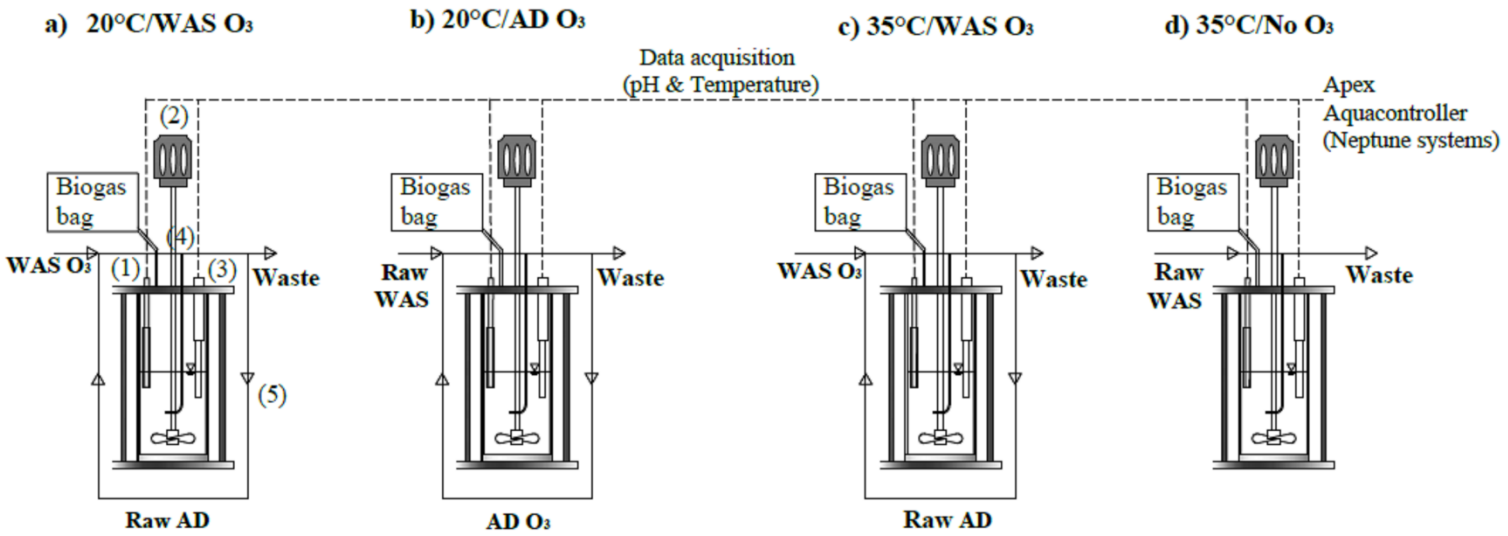

Fig. 5.1. Schematic overview of four anaerobic sequencing batch reactors (ASBRs) operated at a) 20 ℃ and fed with ozonated WAS and raw AD, b) 20 °C and fed with ozonated AD and raw WAS, c) 35 °C and fed with ozonated WAS and raw AD, and d) 35 °C fed with raw WAS (Control). Total reactor volume= 4 L, effective working volume=2 L. WAS-Waste activated sludge, AD-Anaerobic digestate, O_3-ozonated substrate. The following identify the different components of the digesters: (1) pH probe; (2) Mechanical mixer; (3) temperature probe; (4) feeding/wasting line; (5) recirculation line.

5.2.2 Ozone treatment

Ozonation of WAS and anaerobic digestate was conducted in a 3-L ozone contactor vessel equipped with two inlet ports fitted with atomizer nozzles supplying ozone at a pre-determined rate. The ozone was generated using Ultra High Purity 4.3 oxygen (Praxair, Mississauga, Ontario) by an ozone generator (Ozomax, model OZO 3VTTL, Canton de Shefford, QC). The dose of ozone to transfer and respective contact time with the sludge were determined based on chemical oxygen demand (COD) solubilization experiments, to identify the optimum ozone dose using freshly collected WAS from the RAEBL wastewater treatment facility or anaerobic digestate from the St-Hyacinthe full-scale anaerobic digester (Quebec, Canada). To determine whether the initial or background soluble COD (sCOD) level present in the WAS or anaerobic digestate had an effect on the final sCOD concentration during ozonation, the substrate was subjected to three different treatments: (1) washed three times and resuspended in phosphate buffer saline (PBS) (Feng, 2014), (2) aerated overnight, (3) aerated overnight followed by washing 3 times and resuspension in PBS. For anaerobic digestate, an additional experiment, where the samples were mixed with 20% of fresh WAS and was aerated overnight prior to ozonation, was also conducted. The concentrations of the sCOD and VSS were measured after the ozonation experiments. COD solubilization efficiency was determined by plotting measured sCOD values against ozone doses. The optimal ozone dose was determined to be 365 mg O_3/L (corresponding to 0.01 mg O_3/mg VSS) and was used to ozonate the WAS and anaerobic digestate for feeding the reactors. The COD solubilization experiments were performed in triplicates (n=3).

5.2.3 Sampling and analytical methods

To monitor reactor operation, grab digestate samples were collected twice weekly to determine the total soluble and particulate COD fractions (method 5220D) (Rice et al., 2017). For sCOD measurements, digestate samples were centrifuged (20 min. at 4,000×g, Thermo Fisher Sorvall™ ST 16 Centrifuge, USA), followed by filtration of the supernatant using a 0.45-µm membrane syringe filter (Whatman, GE Healthcare, PA, USA). The level of total suspended solids (TSS) and VSS of the digester content was measured using method 2540D (Rice et al., 2017). The concentration of ammonium (NH_4^+-N) was determined using the colorimetric-based Berthelot method in microwell plates, read using the SPECTRAmax® microplate spectrophotometer, and

analyzed by the SOFTmax® PRO software (Molecular Devices, CA, USA) (Rhine et al., 1998). Volatile fatty acid (VFA) concentrations were measured using high-performance liquid chromatography (HPLC) (Agilent 1260 Infinty, USA) equipped with a Bio-Rad Aminex HPX-87H column (7.8 mm i.d. × 300 mm and 9 µm particle size). A solution of 95% H_2SO_4 (0.008N) and 5% Acetonitrile was used as mobile phase for the HPLC analysis. The HPLC was operated under the following conditions: 50 ºC column temperature, 0.8 mL/min flow rate, 80 bar of pressure, and 40 µL injection volume. Detection was performed by UV absorption at 210 nm. The volume of biogas was measured using a pressure meter (F16, CECOMP Electronics, USA). The content of the biogas was analyzed using high-resolution gas chromatography, with a thermal conductivity detector (GC-TCD) (Varian, model CP3800, USA) equipped with a GS-CARBONPLOT column (0.53 mm x 30 m and 3.0 µm film thickness, Agilent Technologies, USA). Samples were injected at a flow rate of 7 mL/min, at an injector and oven temperature of 220 ºC and 35 ºC, respectively. Helium was used as carrier gas at a flow rate of 30 mL/min.

5.2.4 Microbial community analyses

Biomass samples were collected from the four laboratory-scale anaerobic digesters, 2-3 times for each period once the ASBRs were at steady-state. The samples were spun by micro-centrifugation (Thermo Scientific, Sorvall Legend Micro 21R, USA) in 1.5-mL tubes and frozen at −80 °C until time of analysis. Total DNA was extracted using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA), following the manufacturer's instructions. The bacterial and archaeal compositions were studied by PCR amplifying the V6-V8 region of the 16S rRNA gene using the common forward primer 926b_F and reverse primer 1392b_R (Engelbrektson et al., 2010), followed by sequencing the pooled amplicons using the 2×300 bp paired-end sequencing reaction on an Illumina MiSeq 300 platform (Illumina, USA) at the McGill University and Génome Québec Innovation Centre (Montreal, QC). Details on the primer sequences, PCR reaction mixtures, and PCR thermal cycles are provided in the Supplementary Materials and Table S5.1. The raw sequencing data in the Fastq format were demultiplexed, denoised, and processed using the MUGQIC Amplicon-Seq pipeline (McGill University and Génome Québec Innovation Centre). Demultiplexed and quality filtered sequences were clustered into operational taxonomic units (OTUs) using VSEARCH (Rognes et al., 2016). Taxonomic affiliation and identity assignment of the 16S rRNA data were performed using QIIME based on the Greengenes database

(Caporaso et al., 2010b). Further bioinformatic analyses, such as Alpha (rarefaction curves and diversity indices) and beta (principal coordinate analyses, PCoA) diversity, are described in the Supplementary material.

5.2.5 Statistical analysis

One-way ANOVA was performed to assess the variation between the reactors' performance at different SRTs (10, 15, and 20 days) and temperatures. The analysis was conducted on data generated during the near steady-state sub-periods (i.e. the last 63 days for the reactor operated at SRT=20, SRT=15, and SRT>15d, and final 42 days for the reactor operated at SRT=10 days). These sub-periods were subdivided into 3 time-intervals of 21 and 14 days, respectively. The average measurements for these time-intervals were used as independent variables for the statistical analyses. The ANOVAs on the reactor operational data were performed using Microsoft Excel 2016. Statistical significance of mean differences between reactor conditions was assessed by the Least Significant Difference (LSD) (Tallarida & Murray, 1987). Statistical significance of differences in microbial community compositions between reactor conditions was assessed by an independent ANOVA on the abundances of each of the populations tested (Witt et al., 2012). ANOVAs on population abundances were performed using the Paleontological Statistics (PAST3) software (Hammer et al., 2008a). To determine whether any of the differences between the means are statistically significant, a significance level (α or alpha) of 0.05 was considered.

5.3 RESULTS AND DISCUSSION

5.3.1 Optimum ozone dose

Increasing the ozone concentration resulted in an increase in the sCOD concentration, as shown by the solubilization curves in Figures S5.1b (for WAS) and S5.1d (for anaerobic digestate). For both substrates, the curves comprised a generally linear portion where the release of sCOD was directly proportional to the ozone dose, followed by a plateau where no further increase in sCOD was observed despite an increase in the ozone transferred. Hence, the optimum ozone dose was defined as the highest concentration on the linear portion of the graphs, just before they reached the plateau. For both WAS and anaerobic digestate, the last segment of the linear portion of the curve corresponded to 365 mg/L (or 0.01 g O_3/g VSS_{in}), which was deemed to be the optimum

ozone dose to induce maximum COD solubilization. To determine whether the initial or background sCOD level in either the WAS or digestate had any effect on the final sCOD concentration following ozonation, both matrices were washed with phosphate buffer, and/or aerated overnight, followed by pre-treatment at different ozone doses. The results showed that the difference between the initial and final sCOD was similar for the untreated or pre-treated substrates (washed or aerated) suggesting that the background sCOD level did not affect the solubilization profile for both WAS and digestate.

5.3.2 Digestion performances in function of SRTs

The reactor operated at 35 °C, and when fed with ozonated WAS, showed the highest biogas production, followed by the 20 °C/AD O_3, 20 °C/WAS O_3, and 35 °C/No O_3, respectively (averages per operation period in Fig. 5.2; complete operation data in Fig. S5.2). Thus, at all SRTs and both temperatures (20 °C and 35 °C), ozonation of WAS or digestate led to a higher VSS destruction and biogas production. as compared to the control non-ozonated digester at 35 °C. Further comparing the two reactors operated at 20 °C, ozonating the anaerobic digestate instead of the WAS led to an additional increase in biogas production, suggesting that ozonating the digestate may have a slightly higher performance efficiency (Fig. 5.2b).

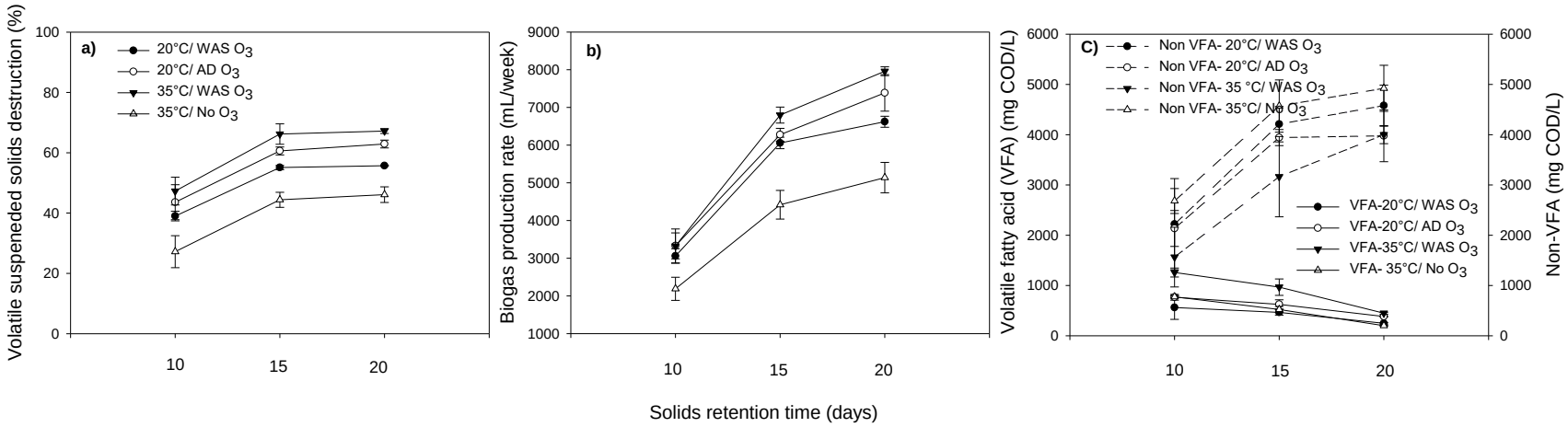

Fig. 5.2. a) Volatile solids destruction, b) Biogas production rate, and c) Volatile fatty acid (VFA) and non VFA COD from anaerobic digestion of WAS and AD at different SRTs. WAS-waste activated sludge; AD-anaerobic digestate; O_3-ozonated substrate. Legend of panel a) also applies to panels b) and c). Error bars indicate standard errors among 3 time-intervals of 21 days for SRT, 15d and 20d, and 3 time-intervals of 14 days for SRT 10d, independent averages were obtained after near-steady-state was reached. The significance of differences between the SRTs is reported in Tables S5.2 and S5.3.

At an SRT of 10 days, VSS destruction in the control reactor (35 °C/No O_3) was only 29% and was lower than the acceptable VSS reduction of 40% for full-scale mesophilic digesters (Rimkus et al., 1982). Marked significant improvements in VSS destruction and biogas production were observed between the 10-d and 15-d SRT periods for all conditions, which was accompanied by significant reduction in VFA concentrations (ANOVA and Least-significant difference, Tables S5.3 & S5.4). These suggest that the extent of disintegration and hydrolysis reactions increased between 10-d and 15-d SRT operations, and that the conversion of VFA to methane also increased. Conversely, changes in VSS destruction between the 15-d and 20-d SRT periods were not significant (Table S5.4). However, the increases in biogas productions were either significant or only marginally not significant at the α-level of 0.05, and the reductions in VFA concentrations were significant (Table S5.4). Consequently, between the 15-d and 20-d SRT periods, the methanogenic conversion of methane seems to have improved slightly, while disintegration and hydrolysis did not change.

For all reactor conditions, the concentrations of soluble COD (Fig. S5.2c) increased with increasing SRTs in a way similar to the VSS destruction (Fig. 5.2a). Consequently, the decrease in VFA concentrations with increasing SRTs (Fig. 5.2c) led to an increase in non-VFA soluble COD (Fig. 5.2c). The nature of the accumulating soluble COD remains unclear. It could include slowly degradable substrates or undegradable compounds.

The lower VSS destruction at low SRT is similar to results obtained by previous groups for mesophilic anaerobic digesters treating raw WAS at 35 °C, and are explained by the limitation of disintegration and hydrolysis (Nges & Liu, 2010; Yuan et al., 2016). Nonetheless, our results clearly show that even at low SRTs, the ozonation treatment enhanced these reactions. The accumulations of VFA at lower SRT for all conditions and with ozone treatment at 35 °C are also in line with the literature. Goel et al. (2003a) observed that the VFA content of digested sludge pretreated with ozone was significantly higher as compared to untreated sludge. In our results, acetic acid, propionic acid, and butyric acid were found to predominate in the reactors, especially those fed with ozonated substrates at low SRT, with the 35 °C/WAS O_3 digester operated at a 10-d SRT exhibiting the highest levels of these three individual VFAs (Fig. S5.3). Similarly, Wijekoon et al. (2011) observed acetic acid and butyric acid to prevail as predominant VFAs in sludge, and Capson-Tojo et al. (2017) suggested that propionic acid accumulation may

predominate during anaerobic digestion of certain complex wastes. Moen et al. (2003) found that the propionate concentration had a five-fold increase when the SRT was reduced from 10 to 4 days, while in this study the average propionate concentration increased by almost 1.5-fold when SRT was decreased from 15 to 10 days.

The interest in varying the SRTs was to determine the optimal SRT, defined as the SRT that achieves 80% of the maximum VSS destruction and biogas yield. Our results suggest that at an SRT of 15 days, the maximum VSS destruction was achieved. Consequently, it appears that the biogas yield approaches the maximum at an SRT of 20 days, and the 15-d SRT yielded at least 85% of the biogas obtained at the 20-d SRT. Based on these results, the 15-d SRT was found to correspond to the optimal loading rate for these reactors and was chosen to perform the HRT and SRT decoupling experiments.

5.3.3 Effect of SRT-HRT decoupling

Decoupling the SRT from the HRT can be achieved by separating the supernatant, thickening the digestate and recycling it into the digester system. The amount and activity of the retained biomass in the thickened, recycled sludge can significantly affect the efficiency of the digestion process (Vanyushina et al., 2012a). The key advantage of such a strategy is the possibility of increasing the SRT without increasing the HRT, and therefore, increasing the degree of volatile solids destruction and biogas production. Based on results presented above, the SRT of 15 days was identified as optimum. Operating the reactors at an SRT greater than the HRT led to an increase in VSS destructions by 14% to 18% over those coupled digesters (SRT=HRT=15 days) (Fig. 5.3a). Vanyushina et al. (2012b) also found that increasing the SRT without increasing the HRT in a thermophilic anaerobic digestion system resulted in a VSS reduction of 68% in the test reactor compared to only 34% in the coupled reactor.

The SRT-HRT decoupling also enhanced the biogas production by 20% to 40% (Fig. 5.3.b), which was accompanied by a reduction in the concentrations of total VFAs and non-VFA COD (Figs. 5.3c and d). The higher level of the non-VFA COD in the coupled reactors (SRT=HRT=15 days) suggests that decoupling circumvented a certain limit to the conversion of soluble COD to methane, which increased the digestion efficiency and biogas production. Vanyushina et al. (2012b) also observed an increase in biogas production by 13% over the control reactor when

operating under a decoupled condition (i.e. at an SRT of 17 days and HRT of 9 days). These results suggest that at least a portion of the non-VFA is composed of slowly degradable compounds. However, the remaining fraction could still be completely refractory. Saktaywin et al. (2005) reported that around 60% of soluble COD generated from ozonation was biodegradable and could be converted to biogas, while the remaining soluble organic matter was refractory.

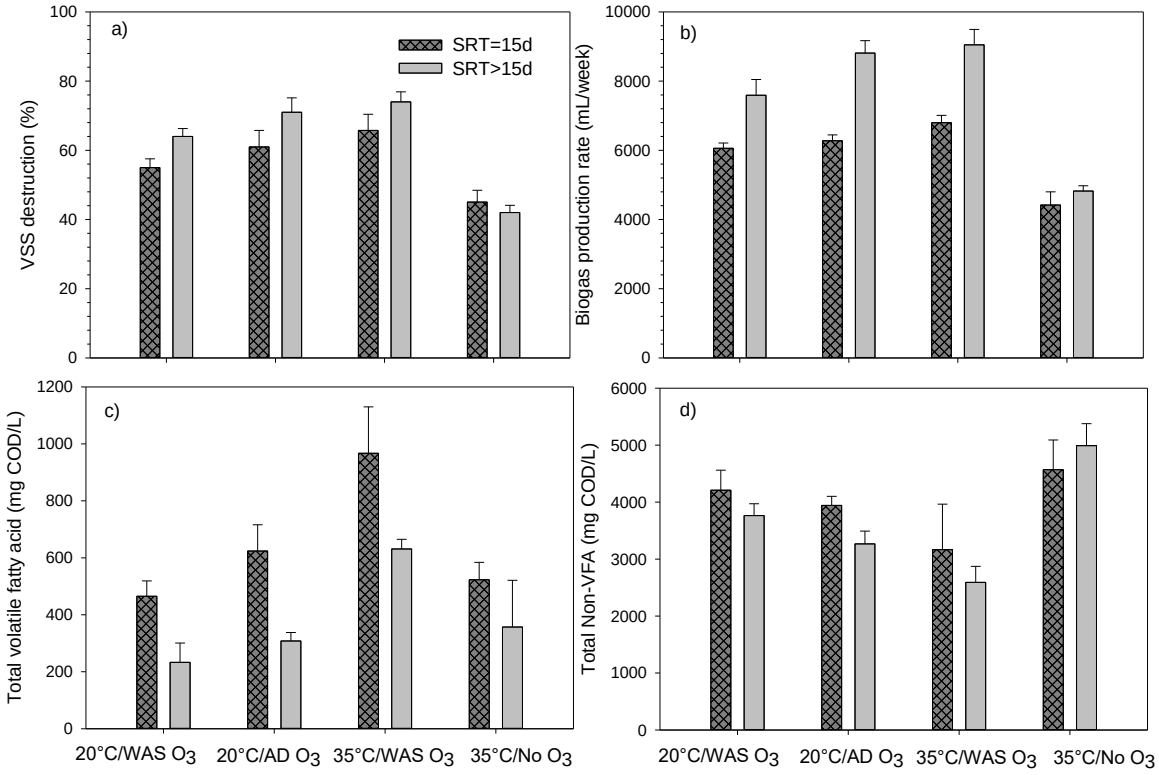

Fig. 5.3. a) Volatile solids destruction, b) biogas production rate, c) Volatile fatty acid (VFA), and d) non-VFA COD from anaerobic digestion of WAS and AD at SRT=15d and SRT>15d. WAS-waste activated sludge; AD-anaerobic digestate; O_3-ozonated substrate. Legend of panel a) also applies to panel b), c) and d). Error bars indicate standard errors among 3 time-intervals of 21 days for SRT 15d and >15d, independent averages obtained after near-steady-state was reached. The significance of differences between the SRTs is reported in Table S5.4. *Note*: For SRT>15d, the level of solids in the three ozonated digesters was maintained at the same level as in the 35 °C/ No O_3 digester. The SRTs for the SRT>15d period were: 15 d for the 35 °C/ No O_3 (Control), 24 d for the 35 °C/ WAS O_3, 19 d for the 20 °C/ WAS O_3 and 21 d for the 20 °C/ AD O_3 reactors.

5.3.4 Microbial community analysis

Biomass samples were periodically collected and analyzed to gain insight into the bacterial and archaeal population dynamics under the different operational configurations. The total number of raw and denoised high-quality reads for *Bacteria* and *Archaea* after the amplicon sequencing is summarized in Table S5.5.

5.3.4.1 Bacterial and archaeal diversity

Rarefaction curves were generated to estimate the microbial diversity of the digesters (Figs. S5.4 & 5.5). Although most samples did not completely achieve the plateau, the high Good's coverage values (>97%) suggested that the amplicon sequencing depth adequately estimated the microbial diversity of the reactors. In general, the bacterial and archaeal species richness, expressed as the number of observed OTUs, was slightly higher in the reactors fed with ozonated WAS than in the one fed with non-ozonated sludge (Tables S5.6 and S5.7, Figs. S5.4 and S5.5). The reactor operated at 20 °C with ozonated digestate had a higher richness and diversity than the digester operated at 20 °C with ozonated WAS. At 35 °C, the digester fed with ozonated WAS resulted in a higher species richness and diversity than the reactor operated at 35 °C with non-ozonated WAS. At the same time, lower SRT in general led to a lower number of OTUs in all the reactors. When the SRT is less than the growth rate, washout of key microorganisms occurs, leading to process failure. Methanogens are considered to be the slowest growing microorganisms in anaerobic digestion systems, and under mesophilic conditions, they can be strongly impacted by low SRTs (Amani et al., 2010; Ho et al., 2014).

The phylogenetic composition and structure of the microbial communities of the anaerobic digesters were visualized by Principal Coordinate Analysis (PCoA) based on the Bray-Curtis distance metric. Analyzing the communities between the reactors at the 15-day SRT, considered as the optimum SRT, revealed subtle differences in both bacterial and archaeal phylogenetic groups. The two reactors operated at 20 °C clustered closely, indicating comparable bacterial population structures (Fig. 5.4a). Conversely, the reactor operated at 35 °C and fed with ozonated WAS (35 °C/WAS O_3) clustered distinctly from the other reactors. Similar trends were observed for the archaeal populations (Fig. 5.4b). Based on the abovementioned results, it is likely that such

variation in community composition and structure are aresultof the different operational conditions (temperature, SRT and ozonation).

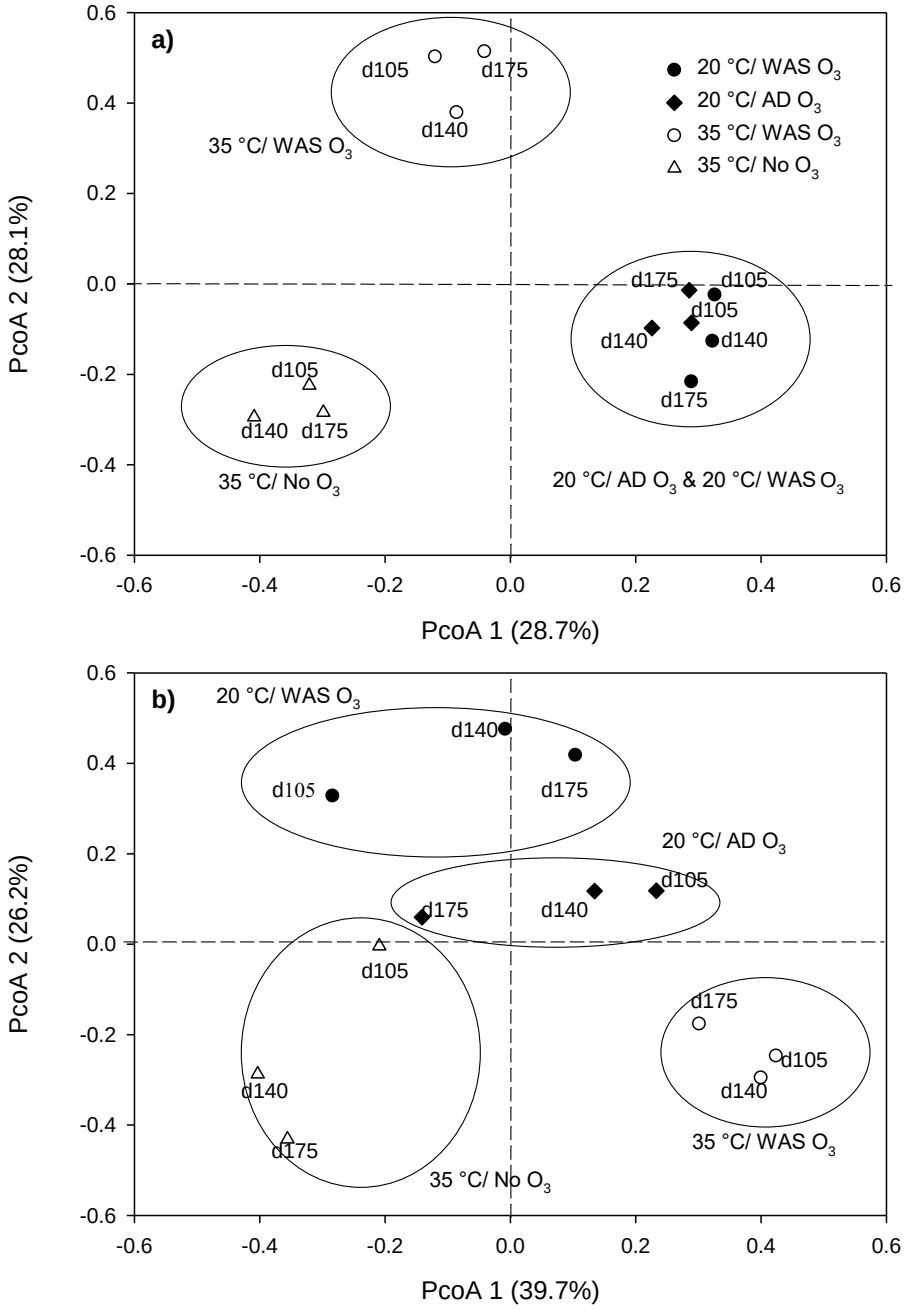

Fig. 5.4. Principal coordinate analysis (PCoA) of bacterial (a) and archaeal (b) *16S rRNA* gene amplicon Illumina sequencing data for anaerobic digestate samples at SRT of 15 days. Ordination was performed based on Bray-Curtis distance and OTUs were defined at 3% similarity.

5.3.4.2 Taxonomic composition of metagenomes

At phylum level, most of the bacterial sequences were affiliated with the *Firmicutes and Bacteroidetes* phyla (Fig. S5.6), similar to previously reported data for anaerobic sludge digesters (Nelson et al., 2011; Riviere et al., 2009a). For the archaeal populations, *Euryarchaeota*, which contains methanogens, they were found to be the dominant phylum in all the reactors with readings of > 98.4% (Fig. S5.7); this is also in line with previous studies on anaerobic digestion (Sun et al., 2015).

5.3.4.3 Microbial dynamics across reactors

The composition and relative proportion of taxonomic groups at the genus level revealed major differences in the bacterial and archaeal populations under the different operational conditions by focusing on the 8 most abundant bacterial genera (representing ~75% of the bacterial reads; Fig. 5.5). By increasing the SRT, the abundance of *Clostridium* (phylum *Firmicutes*) in all digesters under the ozonated feeding regime was decreased, while the reactor operated under conventional configuration (35 °C/No O_3) contained a lower abundance of this group and the opposite trend was observed (Fig. 5.5). Similar observations were obtained for *Candidatus Cloacamonas* in the ozonated fed digesters, where an increase in the SRT, from 10 to 20 days, led to a significant decrease in their abundance, while no significant change was noticed for the 35 °C/No O_3 digester. In the control reactor (35 °C/No O_3), a similar trend was observed for *Anaerolinea*, where an increase in SRT resulted in a decrease in the abundance, while a change in digester configuration and SRT did not have a notable impact on the relative abundance of *Anaerolinea* for the ozonated digesters.

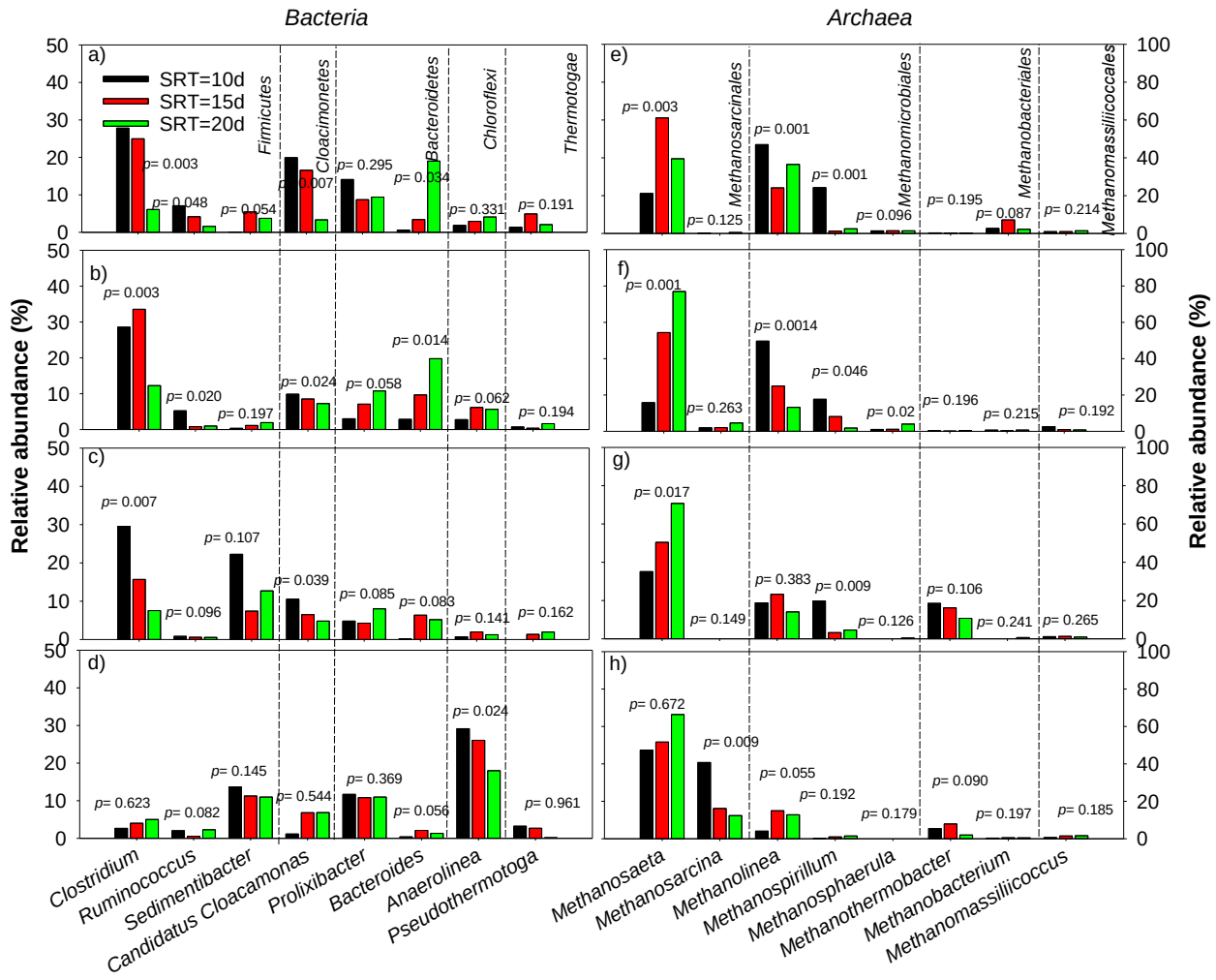

Fig. 5.5. Distribution of the eight (8) most abundant *Bacterial* and *Archaeal* genera in the four ASBRs operated at different SRTs. The genera belonging to the same phylum are grouped together. The height of each bar represents their relative abundance. Digester a & e) 20 °C/ WAS O_3, b & f) 20 °C/ AD O_3, c & g) 35 °C/ WAS O_3, and d & h) 35 °C/ No O_3. Phylogenetic groups accounting for ≥2% of all classified sequences were considered. Each bar represents averages of the following days: SRT = 15 days: Day 105, 140, and 175; SRT = 10 days: Day 210 and 245; and SRT =20 days: Day 281 and 316. The *p* value presented above each group represents ANOVA between SRTs (α=0.05).

Other abundant genera belonging to the *Firmicutes* phylum were *Sedimentibacter*. *Sedimentibacter* were found to be significantly more abundant in digesters operated at 35 °C (35

°C/WAS O_3 and 35 °C/No O_3), and they did not change significantly with SRT. Conversely, *Bacteroides* were present at a higher abundance when the digesters were operated at lower temperature. The contrasting profiles observed may be due to the temperature difference (20 °C and 35 °C) at which the reactors were operated.

As presented above, SRTs and HRTs were decoupled in the last experimental section to adjust the concentration of solids in the ozonated reactors to the same level as the Control reactor (35 °C/No O_3). The SRT of the control reactor was not decoupled and was maintained at 15 days throughout the SRT/HRT decoupling experiment. Thus, in order to have the same level of solids as the 35°C/ No O_3 digester, the SRT of the ozonated digesters was increased ($SRT_{ozonated} = SRT_{35°C/\ No\ O3} \times VSS_{35°C/\ No\ O3} / VSS_{ozonated}$). Increasing the SRT (> 15 days) while maintaining the HRT at 15 days followed the same trend as previously observed when the SRTs was increased from 10 to 20 days.

Members of the *Firmicutes* phylum, such as the genus *Clostridium*, *Ruminococcus,* and *Sedimentibacter,* are fermenting bacteria, which are involved in bio-hydrogen production under anaerobic conditions (Kapdan & Kargi, 2006). The hydrogen is utilized by methanogens, via the hydrogenotrophic pathway, to produce methane. Previous studies have also highlighted the relative abundant increase with decreasing SRT in anaerobic digesters of *Clostridium* (Vanwonterghem et al., 2015). Such increases may be correlated with an increased production and accumulation of acetate at low SRT, as was also observed by Schnurer et al. (1996) and Hattori (2008), considering that *Clostridium* is a classical acid producer and usually ferments glucose into butyrate, acetate, carbon dioxide, and molecular hydrogen. *Sedimentibacter* is a hydrogen-producing acetogenic bacterium capable of producing hydrogen and acetic acid in the hydrogen and methane coproduction process (Jia et al., 2016). Studies have shown that *Sedimentibacter* were abundant at elevated temperatures, especially at 35-50 °C, which could accelerate hydrolytic activity for lipids, proteins, and polymeric carbohydrates (Lin et al., 2016; Nelson et al., 2011; Sundberg et al., 2013). The presence of *Ca.* Cloacamonas, *a* bacteria producing H_2 and CO_2 from formate (Pelletier et al., 2008), may explain the accumulation of VFAs in these reactors, especially during the increase of the SRT from 10 days to 20 days. *Ca.* Cloacamonas principally uses fermentation processes as its carbon and energy sources. This suggest the strong selective pressure of SRT.

Bacteroides are hydrolytic bacteria which are involved in the hydrolysis step of anaerobic digestion (Post et al., 1967). The relative abundance of *Bacteroides* increased with increasing SRTs at lower temperatures. However, previous studies have shown that they have fast growth rates and are less sensitive to changes in environmental conditions (Li, 2013). While *Anaerolinea*, belonging to the phylum *Chloroflexi*, was present at higher abundance in the reactor operated at 35 °C with non ozonated substrate, low abundance was observed for the digesters fed with ozonated substrates. *Anaerolinea* is known to grow fermentatively, with a range of carbohydrates and yeast extracts as substrates. Narihiro and Sekiguchi (2007) suggested that members of this genus might play a main role in the primary degradation of carbohydrates and cellular materials (such as amino acids) in methanogenic digestion processes. Previous studies have reported the existing link between microbial community dynamics and operational conditions, such as temperature and SRT of anaerobic digesters (Loreau et al., 2001; Vanwonterghem et al., 2015). Such differences are potentially related to the system performance and the stability of the anaerobic digesters; the change in operational configurations seems to have altered the microbial community composition and structure, which, in turn, impacted on the performance of the digesters. Thus, in the digesters operated under the ozonated feeding regime, the significant change in the relative abundance of these genera might be due to a change in the nature of the feed, where simpler substrates such as sugars were available, while for the conventional anaerobic digester, the higher abundance of *Anaerolinea* might be due to a higher concentration of cellular materials (such as amino acids) present in the substrate.

The archaeal communities in all the four digesters belonged mainly to the *Euryarchaeota* phylum represented by a high proportion of acetoclastic and hydrogenotrophic methanogens. *Methanosaeta* has been reported to dominate anaerobic digesters (Karakashev et al., 2005a; Walter et al., 2012). The relative abundance of *Methanosaeta* was significantly increased from SRT of 10 days to SRT of 20 days in digesters operated with ozonated substrate, while no significant change was observed for the Control digester (35 °C/ No O_3) (Fig. 5.5).

Hydrogen is used as an electron donor to form methane by hydrogenotrophic methanogens (Demirel & Scherer, 2008). *Methanolinea*, belonging to the hydrogenotrophic order *Methanomicrobiales*, has been reported to be a dominant H_2/CO_2-using methanogenic archaeon (Imachi et al., 2008). Operating the anaerobic digesters at lower temperature (20 °C) resulted in a

higher abundance of *Methanolinea* than in the 35 °C digesters. Increasing the SRT from 10 to 20 days led to a significant decrease in the relative abundance of this genus in the 20 °C/ WAS O_3 and 20 °C/ AD O_3 digesters, while no significant change was observed for the 35 °C reactors. Another hydrogenotrophic methanogen which was in high abundance in all of the digesters was *Methanospirillum,* also belonging to the order *Methanomicrobiales*. The use of ozonated feed was found to enrich *Methanospirillum* over non-ozonated substrates. The results also found that in all ozonated digesters, an increase in SRT led to a significant decrease in the relative abundance of *Methanospirillum*. The high VFA levels resulting from acidogenesis reactions under ozonated conditions appeared to favor the growth of *Methanospirillum,* which has been shown to be important H_2-consuming partners in VFA-degrading co-cultures (McInerney et al., 2008; Stams et al., 2012).

A shortening of the SRT, hence, led to a shift in the methanogenic pathway from acetoclastic, involving *Methanosaeta* to hydrogenotrophic reactions by *Methanolinea* and *Methanospirillum*. Washout of the slow-growing *Methanosaeta* and a shift to hydrogenotrophic methanogenesis at reduced SRT has also been reported by previous studies (De Vrieze et al., 2012; Ziganshina et al., 2014). According to Ju et al. (2017), the microbial community distribution in anaerobic digesters is proportioned as a response to operational fluctuations such as SRT by species rearrangement to adjust to environmental stress. Altering the SRT, thus, induced a change in functional organization of the community and shifted the methanogenic pathway accordingly, as observed in the present study. Marzorati et al. (2008) reported that effective VFA accumulation at low SRTs induces adjustment of the community and a shift in dominance of key populations to preserve functionality of the anaerobic fermentation process, but with fragile resistance to further environmental perturbations.

No significant change was observed between the archaeal community structures under the SRT-HRT decoupled and coupled conditions for all the reactors (Fig. 5.6). Hence, maintaining high biomass retention in the system to compensate for slower kinetics, while decreasing biomass washout, appeared to have mainly influenced the bacterial rather than the archaeal populations. Separation of active solids from the effluent by centrifugation to recycle them back (i.e. the pellet) to the digester may cause separation and loss of communities to the supernatant, to some extent. Studies have highlighted the strong difference in adhesion capacity between bacteria and archaea

in flocs of granulated structures in anaerobic digestion systems (Gagliano et al., 2017; Habouzit et al., 2011). Archaea presumably has a higher adhesion and granulation efficiency, together with a higher tolerance to turbulence and shear (Grumezescu & Holban, 2017). This may, in part, explain the resilience of the archaeal community during the liquid/solid phase separation during centrifugation and recycling back into the digesters under the decoupled condition.

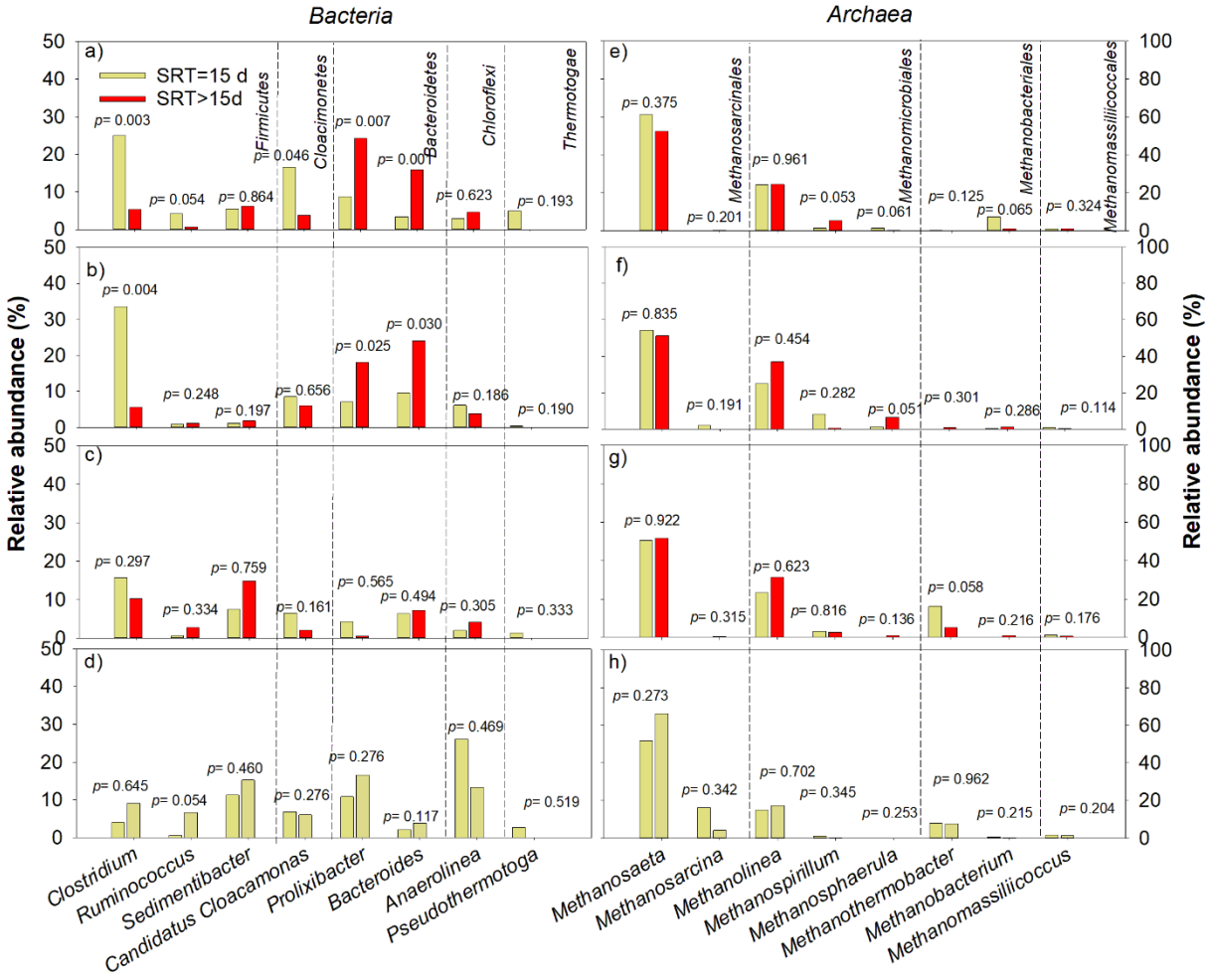

Fig. 5.6. Effect of SRT-HRT decoupling on the distribution of the eight (8) most abundant Bacterial and Archaeal genera in the four ASBRs operated for SRT=15 days and SRT>15 days. The genera belonging to the same phylum are grouped together. The height of each bar represents their relative abundance. Digesters a & e) 20 °C/ WAS O_3, b & f) 20 °C/ AD O_3, c & g) 35 °C/ WAS O_3, and d & h) 35 °C/ No O_3 (the second bar corresponds to phase two when the

other reactors have an SRT>15d). Phylogenetic groups accounting for ≥2% of all classified sequences were considered. Each bar represents average of the following days: SRT = 15 days: Day 105, 140, and 175 (Period 3), and SRT >15 days (Period 5): Day 350 and 385. The *p* value presented above each group represents the ANOVA between SRTs (α=0.05).

5.4 CONCLUSIONS

In this study, we assessed the performance of anaerobic digestion combining sludge ozonation at low mesophilic temperature (20 °C) in comparison to conventional anaerobic digestion of raw sludge at 35 °C. Implementation of such a hybrid system led to a higher digester performance at 20 °C, with an enhanced VSS reduction by 20% and biogas production by 29% than conventional anaerobic digestion at 35 °C with untreated sludge. Ozonating the anaerobic digestate rather than the WAS increased the VSS reduction and biogas production by almost 10%, showing that the point of ozonation is an important factor to consider when implementing low temperature anaerobic digestion. Variation in SRT clearly affected the reactor performance due to accumulation of VFAs at low SRTs. Decoupling the SRT from the HRT significantly improved the VSS reduction and methane yield at low temperature. The microbial community composition and dynamics were also evaluated and showed clear shifts with variation in SRT. Either the change in growth rates (related to the SRTs) or the accumulation of VFA affected the community assembly, but further work will be needed to elucidate the exact mechanisms.

5.5 ACKNOWLEDGEMENTS

We thank the staffs of the RAEBL WRRF who assisted us in collecting raw WAS samples, and the St-Hyacinthe WWRF for providing us with anaerobic digestate inoculum for the reactor start-up. The contribution of the Centre National en Électrochimie et en Technologies Environnementales (CNETE) in Shawinigan, Quebec (Canada), is especially acknowledged for performing the VFA analysis. This study was funded by the Natural Sciences and Engineering Research Council (NSERC) of Canada, Air Liquide Canada and the Régie d'Assainissement des Eaux du Bassin LaPrairie (RAEBL). The project was supported by the Mathematics of Information Technology and Complex Systems (MITACS) of Canada. Z. B. was partly supported by the McGill Engineering Doctoral Award (MEDA). The findings of this research work were presented at the 4th IWA Specialized International Conferences on "Ecotechnologies for Wastewater

Treatment (ecoSTP2018), which was held in London, Canada from 25-28 June 2018. The fruitful discussion therein is also acknowledged.

5.6 SUPPORTING INFORMATION

Table S5.1. Primer sequences and PCR thermal cycles of PCR 1 and PCR2

Primers		Primer Sequence (5'CS--3'CS)	Optimized PCR thermocycling programs
PCR1 (Engelbrektson et al., 2010)	926b_F	AAA CTY AAA KGA ATT GRC GG	Initial denaturation at 91°C for 3 min; then 25 cycles of: 95°C for 30 s, 62.2°C for 45 s, and 72°C for 90 s; then final extension at 72°C for 10 min.
	1392b_R	ACG GGC GGT GTG TRC	
PCR2	Uniprimer1	AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCC CTA CAC GAC	Initial denaturation at 94°C for 3 min; then 25 cycles of: 94°C for 45 s, 72.9°C for 30 s, and 72°C for 90 s; then final extension at 72°C for 10 min.
	Uniprimer2	CAA GCA GAA GAC GGC ATA CGA GAT CGA TGT GTG ACT GGA GTT C	

Microbial community analyses

Each 50µl of PCR reaction mixture contained 0.5 µM of forward and reverse primer, 1 × 5X Bioline PCR colorless buffer (Taunton, MA, USA), 2.75 mM $MgCl_2$, 250 µM dNTP (each), 12 ng/mL DNA template and 2.5 units Bioline Taq DNA Polymerase (Taunton, MA, USA) in UltraPure™ DNase/RNase-Free Distilled Water (Invitrogen, Carlsbad, USA). The PCR amplicons were purified using the QIAGEN QIAquick® PCR Purification Kit (Hilden, Germany) to remove primers, enzyme, buffer and primer-dimers. A second PCR was performed using primers tagged with sequencing adapters and barcodes (NEXTflex™ DNA Barcodes, Bioo Sicientific) for sample identification following multiplexed sequencing. The PCR mixture was allowed to undergo an initial denaturation at 94 °C for 3 min, followed by 25 cycles of denaturation at 94 °C for 45 s, and annealing at 72.9 °C for 0.5 min. Extension was performed at 72 °C for 90 s before a final extension at 72 °C for 10 min. The resulting amplicons were purified using the QIAGEN QIAquick® PCR Purification Kit (Hilden, Germany), and individually quantified using the Quant-iT™ PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, USA). Forty-four purified amplicons were normalized and pooled for high throughput Illumina sequencing.

Bioinformatic analysis

The raw sequencing data in Fastq format were demultiplexed, denoised and processed using the MUGQIC Amplicon-Seq pipeline (McGill University and Génome Québec Innovation Centre). Primer trimming was performed to remove primer sequences from the sequencing reads to avoid potential interference. The paired-end reads devoid of primer sequences were merged based on mapped consensus positions using FLASh (Magoč & Salzberg, 2011). Sequence reads with at least 1 "N" base were discarded. A reference-based chimera detection using the GOLD database was performed by UCHIME (Edgar et al., 2011), and removed. Demultiplexed and quality filtered sequences were clustered into operational taxonomic units (OTUs) using VSEARCH (Rognes et al., 2016). OTUs were defined based on a sequence identity threshold of 0.97 which is related to species level (Islam et al., 2015). Taxonomic affiliation and identity assignment of the 16s rRNA data were performed using QIIME based on the Greengenes database (Caporaso et al., 2010b). Multiple alignment of representative OTUs was conducted with PyNAST (Caporaso et al., 2010a). Alpha (rarefaction curves and diversity indices) and beta (principal coordinate analyses, PCoA) diversity analyses were performed using QIIME (Caporaso et al., 2010b), *BiodiversityR* package of the R-software (version 3.2.1), and Paleontological statistics (PAST3) (Hammer et al., 2008a). Principal coordinate analysis (PCoA) was conducted based on Bray-Curtis index. The relative distances of all points represent the relative dissimilarities of the samples according to the Bray Curtis index. The percentage of total variation explained by each PCoA axis is shown in the parentheses. PCoA is a rotation of the original data matrix, and can be defined as a projection of samples onto a new set of axes, such that the maximum variance is projected along the first axis, the maximum variation uncorrelated with axis 1 is projected on the second axis, the maximum variation uncorrelated with the first and second axis is projected on the third axis, etc. The cosine values between the arrow links indicate their correlation.

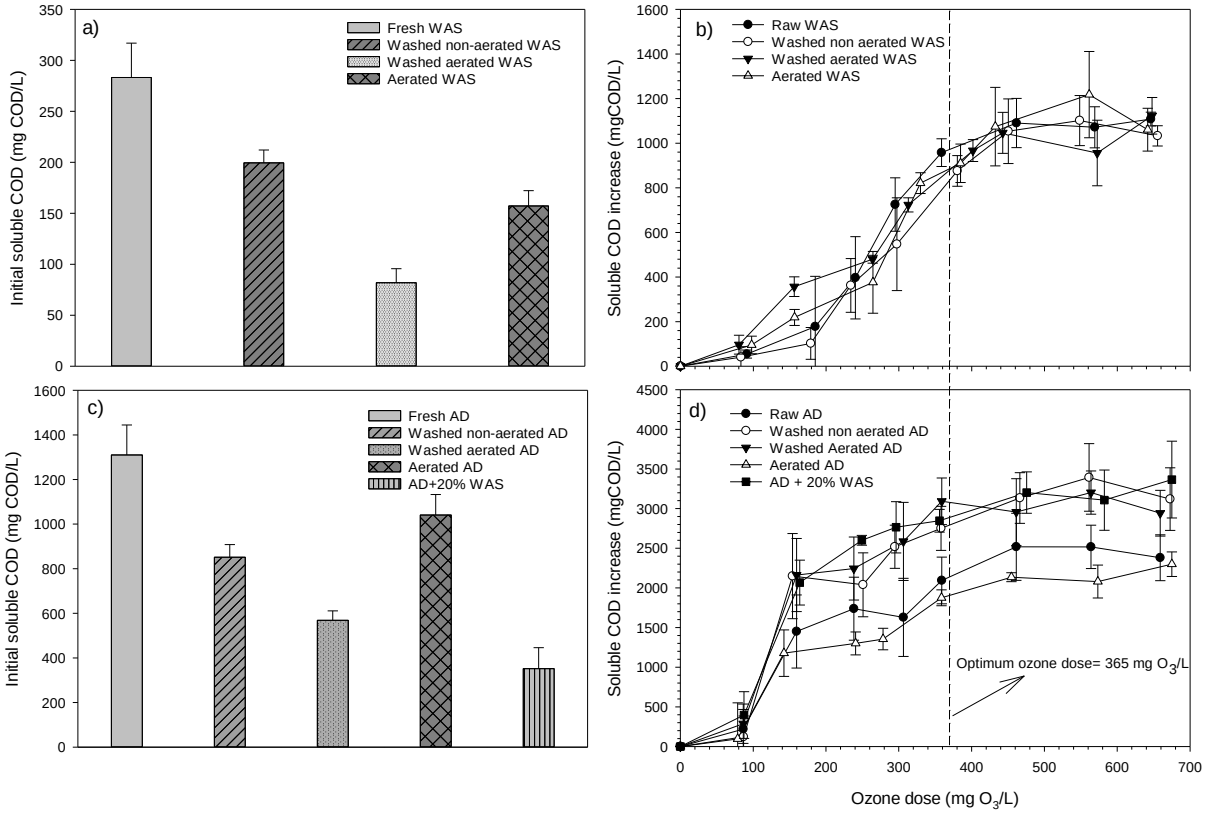

Fig. S5.1. Solubilization of waste activated sludge (WAS) and anaerobic digestate (AD) at different ozone doses. Each point on the curve represents an average of 3 replicates (error bars indicate standard errors; n=3). The samples used for the solubilization experiment contained a VSS concentration of ~30,000 and 20,000 mg/L for WAS and AD, respectively. For panel a and c, the Y axis shows the initial soluble COD at ozone dose of 0, and in panels b and d the Y axis is the increase in sCOD concentration (sCOD at ozone dose (t) - sCOD at ozone dose (0)).

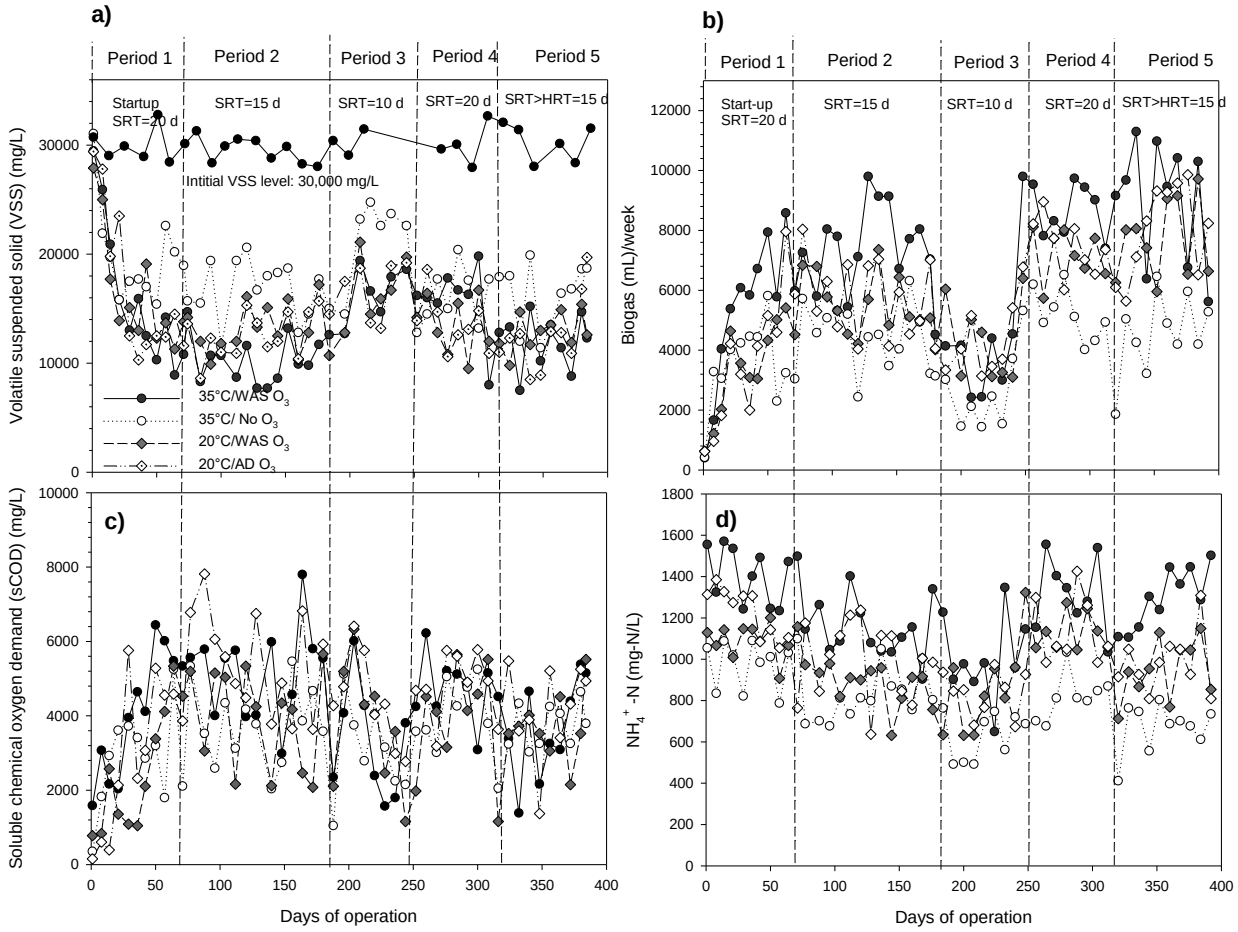

Fig. S5.2. VSS concentration (a), biogas production rate (b), Soluble COD concentration (c), and NH_4^+-N concentration (d), during anaerobic digestion of WAS and digestate at different SRTs. Period 1: start-up period- SRT=20 days, Period 2: Decreasing SRT from 20 to 15 Period 3: decreasing SRT to 10 days, Period 4: SRT=20 days, Period 5: Decoupling of SRT and HRT in the ozonated reactors. The SRT for the 35 °C/ WAS O_3, 35 °C/ No O_3, 20 °C/ WAS O_3, and 20 °C/ AD O_3 reactors in Period 5 was 24, 15, 19 and 21 days, respectively. Average values were calculated among 3 time-intervals of 21 days for SRT 15d and 20d and 3 time-intervals of 14 days for SRT 10d, independent averages obtained after near-steady-state was reached.

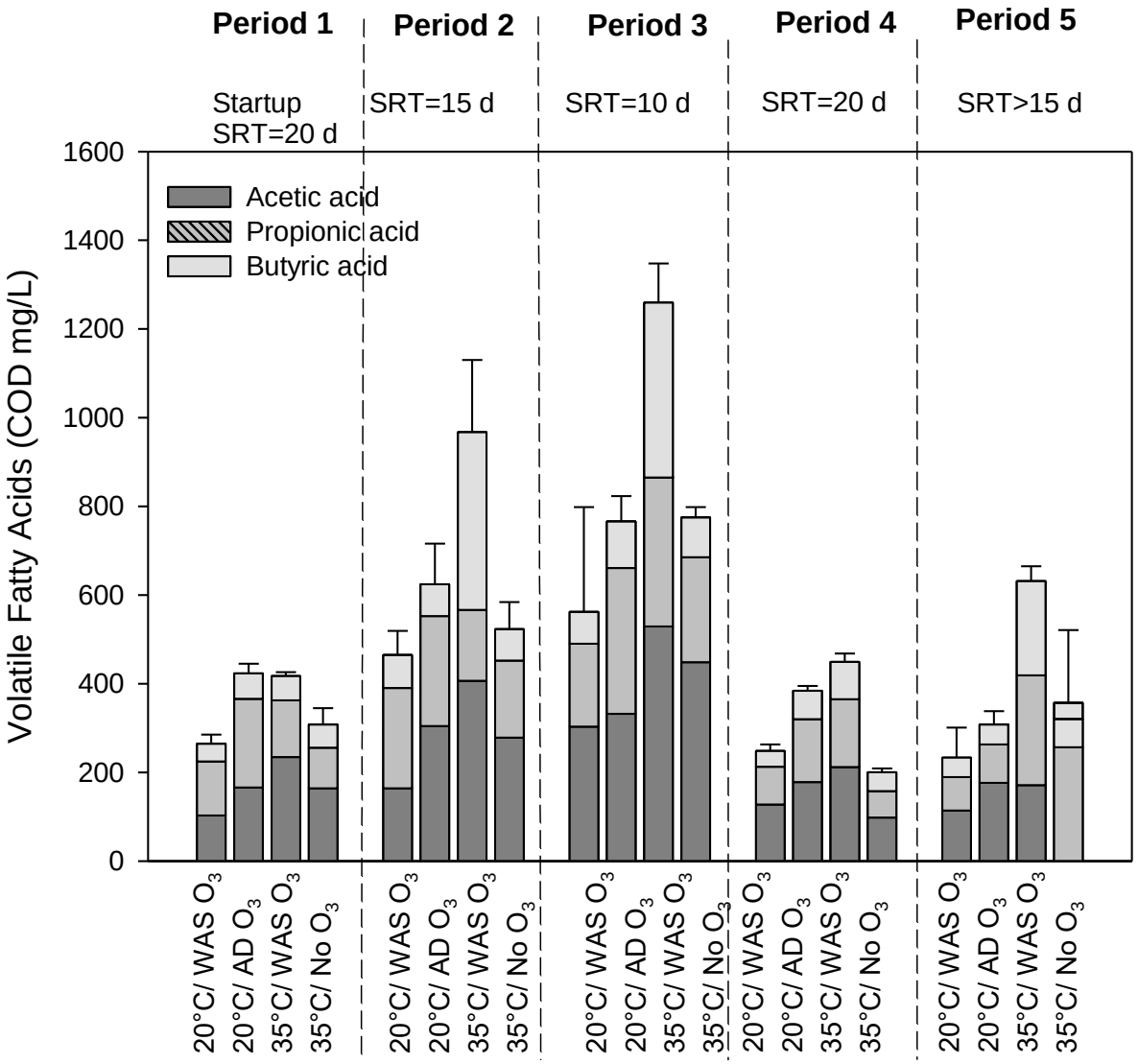

Fig. S5.3. Level of volatile fatty acids (VFAs) in the anaerobic digesters at different SRTs. Each point on the graph represents an average of 3 replicates. Error bars indicate standard error (n=3). Period 1: start-up period- SRT=20 days, Period 2: Decreasing SRT from 20 to 15, Period 3: decreasing SRT to 10 days, Period 4: SRT=20 days, Period 5: Decoupling of SRT and HRT in the ozonated reactors. The SRT for the 35 °C/ WAS O_3, 35 °C/ No O_3, 20 °C/ WAS O_3, and 20 °C/ AD O_3 reactors in Period 5 was 24, 15, 19 and 21 days, respectively.

Table S5.2 ANOVA *p* values testing differences between periods at different SRTs (10, 15 and 20 days) of operation parameter values.

Reactors[1]	Operation Parameters			
	Biogas Production (mL/week)	VSS reduction (%)	VFA (mg COD/L)	Non-VFA (mg COD/L)
20 °C/ WAS O_3	0.00001	0.00004	0.01119	0.04666
20 °C/AD O_3	0.00052	0.01589	0.00005	0.01492
35 °C/ WAS O_3	0.00008	0.00927	0.00001	0.04341
35 °C/ No O_3	0.00308	0.02018	0.00220	0.02743

[1] Note that one ANOVA was computed for each rector and parameter value combination.

Table S5.3 Least significant difference for reactors operated at SRT of 10, 15 and 20 days.

Reactors	**Biogas Production (mL/week)**			
	LSD[1]	$\lvert Ave_{20}-Ave_{15}\rvert$ [2]	$\lvert Ave_{20}-Ave_{10}\rvert$ [2]	$\lvert Ave_{15}-Ave_{10}\rvert$[2]
20 °C/ WAS O_3	570.3	563.2	**2998.5**	**3561.7**
20 °C/AD O_3	1237.2	1112.7	**2946.3**	**4059.0**
35 °C/ WAS O_3	1021.1	**1163.1**[3]	**3472.1**	**4635.2**
35 °C/ No O_3	1267.4	719.8	**2229.2**	**2949.0**
	VSS reduction (%)			
	LSD	$\lvert Ave_{20}-Ave_{15}\rvert$	$\lvert Ave_{20}-Ave_{10}\rvert$	$\lvert Ave_{15}-Ave_{10}\rvert$
20 °C/ WAS O_3	0.0357	0.0061	**0.1613**	**0.1674**
20 °C/AD O_3	0.1221	0.0239	**0.1696**	**0.1935**
35 °C/ WAS O_3	0.1153	0.0106	**0.1883**	**0.1989**
35 °C/ No O_3	0.1278	0.0167	**0.1722**	**0.1888**
	VFA (mg COD/L)			
	LSD	$\lvert Ave_{20}-Ave_{15}\rvert$	$\lvert Ave_{20}-Ave_{10}\rvert$	$\lvert Ave_{15}-Ave_{10}\rvert$
20 °C/ WAS O_3	172.1	**216.4**	97.0	**313.5**
20 °C/AD O_3	74.4	**240.3**	**141.1**	**381.5**
35 °C/ WAS O_3	109.6	**517.8**	**225.7**	**743.5**
35 °C/ No O_3	222.5	**322.4**	**252.1**	**574.6**
	Non-VFA (mg COD/L)			
	LSD	$\lvert Ave_{20}-Ave_{15}\rvert$	$\lvert Ave_{20}-Ave_{10}\rvert$	$\lvert Ave_{15}-Ave_{10}\rvert$
20 °C/ WAS O_3	1900.8	369.2	**1989.1**	**2358.3**
20 °C/AD O_3	1203.0	35.5	**1807.1**	**1842.6**
35 °C/ WAS O_3	1820.2	836.5	1599.2	**2435.7**
35 °C/ No O_3	1581.8	352.0	**1888.4**	**2240.4**

[1]LSD= Least significant difference.

[2]Ave: Average; $\lvert Ave_{20}-Ave_{10}\rvert$: Absolute difference between the average at SRT of 20 days and SRT of 10 days.

[3]Differences larger than the LSD were considered significant at α=0.05. They are noted in bold.

Table S5.4 ANOVA results for reactors operated at SRT=15 days and SRT>15 days

Reactors	*p* Value			
	Biogas Production (mL/week)	VSS reduction (%)	VFA (mgCOD/L)	Non-VFA (mgCOD/L)
20 °C/ WAS O_3	0.033	0.022	0.012	0.288
20 °C/AD O_3	0.003	0.002	0.019	0.056
35 °C/ WAS O_3	0.010	0.008	0.017	0.549
35 °C/ No O_3	0.381	0.628	0.080	0.561

Note: SRT>15 days: Decoupling of SRT and HRT in the ozonated reactors. The SRT for the 35 °C/ No O_3 reactor was 15 days.

Table S5.5. Total raw and high-quality amplicon sequencing reads

Biomass samples*	Total raw reads	Total High-quality reads		OTUs **	
		Bacteria	*Archaea*	*Bacteria*	*Archaea*
	8,841,452	3,681,978	779,258	16,694	929

* Total number of samples= 44; ** OUT classification: 97% sequence identity cut-off

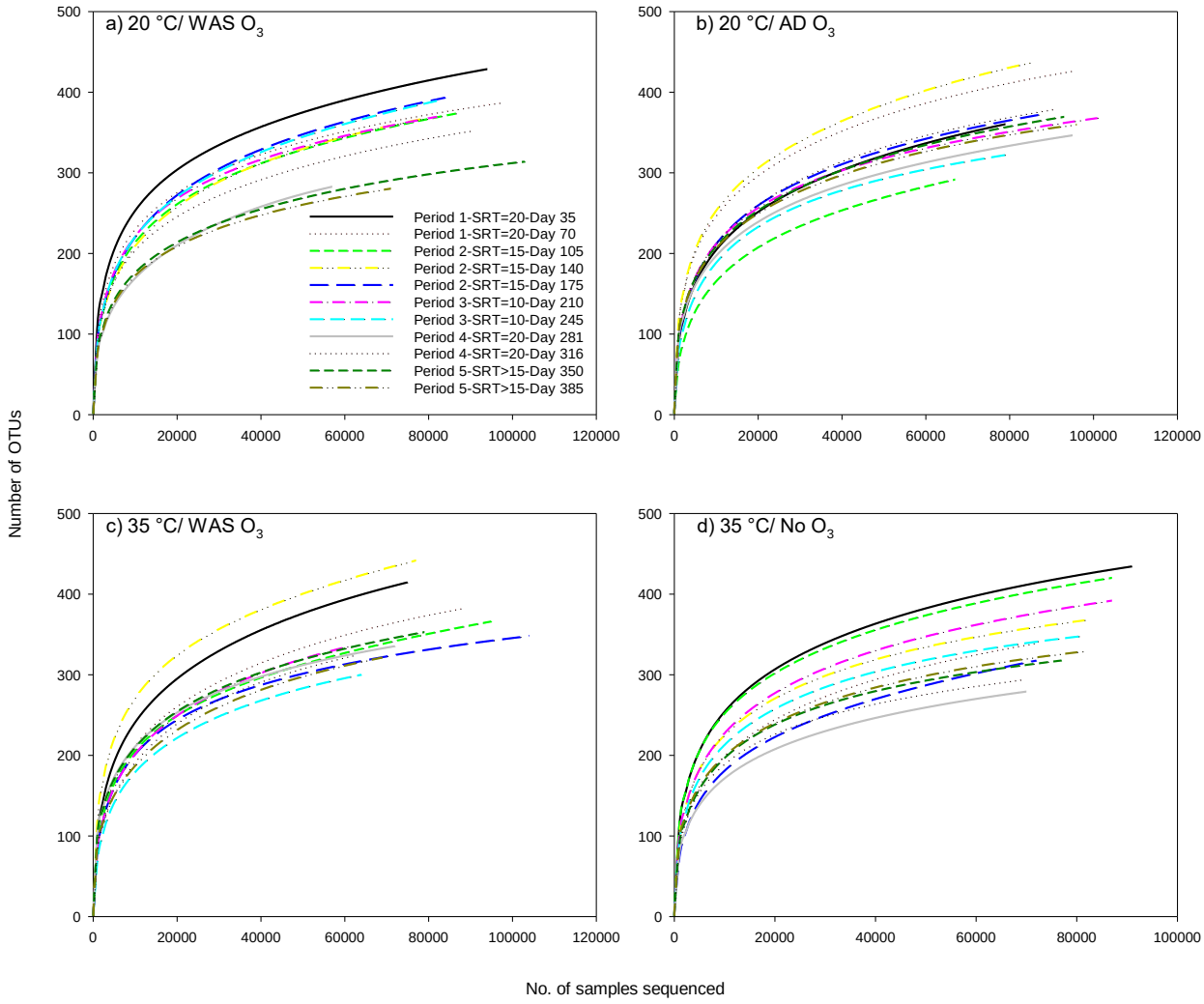

Fig. S5.4. Rarefaction curves of bacterial OTUs defined by 3% sequence variation in anaerobic digestate samples for (a) 20 °C/ WAS O_3 (b) 20 °C/ AD O_3 (c) 35 °C/ WAS O_3 (d) 35 °C/ No O_3. Legend in panel a) applies to the other panels as well.

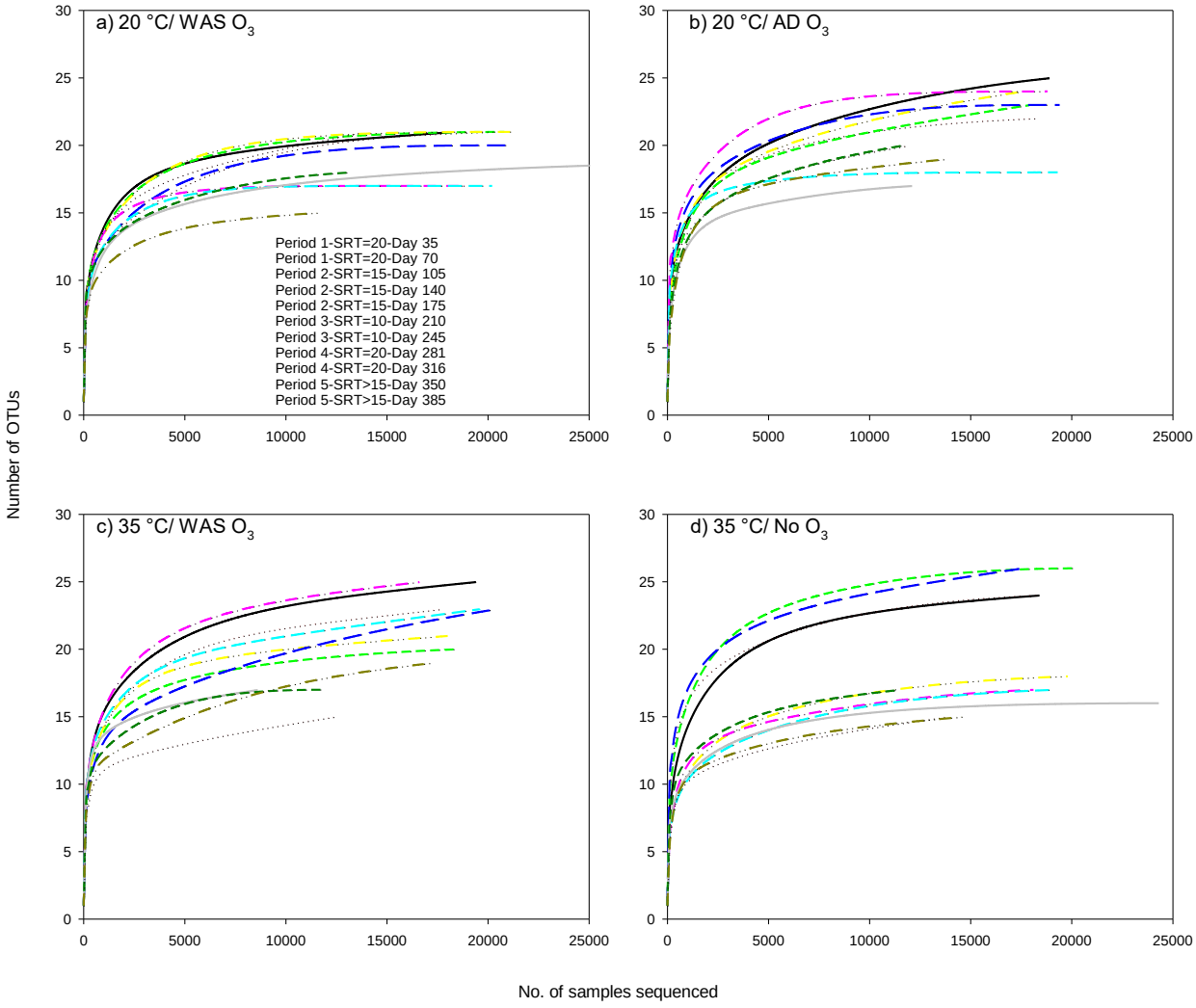

Fig. S5.5. Rarefaction curves of archaeal OTUs defined by 3% sequence variations in anaerobic digestate samples for (a) 20 °C/ WAS O_3 (b) 20 °C/ AD O_3 (c) 35 °C/ WAS O_3 (d) 35 °C/ No O_3. Legend in panel a) applies to the other panels.

Table S5.6 Alpha diversity of bacterial populations in the four anaerobic digesters.

Reactor	Period	SRT (days)	Days	Diversity Indices			
				Simpson	Shannon	Chao-1	Evenness
20 °C/ WAS O_3	1	Start-up/20 days	35	0.95	3.64	513	0.09
			70	0.93	3.44	447	0.08
	2	15	105	0.89	3.09	480	0.06
			140	0.88	3.05	437	0.06
	3	10	175	0.88	2.98	518	0.05
			210	0.87	2.90	450	0.05
			245	0.83	2.64	521	0.04
	4	20	281	0.88	2.90	366	0.06
			316	0.79	2.54	323	0.05
	5	>15	350	0.88	2.81	328	0.06
			385	0.90	3.22	421	0.07
20 °C/ AD O_3	1	Start-up/20 days	35	0.90	3.07	455	0.06
			70	0.91	3.36	498	0.07
	2	15	105	0.79	2.26	407	0.03
			140	0.88	3.20	538	0.06
	3	10	175	0.86	2.85	458	0.05
			210	0.89	3.05	453	0.06
			245	0.85	2.69	444	0.05
	4	20	281	0.91	3.30	452	0.07
			316	0.92	3.37	411	0.08
	5	>15	350	0.89	3.15	449	0.07
			385	0.89	3.20	450	0.06
35 °C/ WAS O_3	1	Start-up/20 days	35	0.94	3.43	522	0.07
			70	0.93	3.27	455	0.07
	2	15	105	0.93	3.29	516	0.07
			140	0.94	3.62	580	0.08
	3	10	175	0.91	3.13	400	0.07
			210	0.85	2.66	399	0.04
			245	0.82	2.41	362	0.04
	4	20	281	0.93	3.51	438	0.09
			316	0.92	3.28	384	0.08
	5	>15	350	0.95	3.68	398	0.12
			385	0.94	3.41	406	0.09
35 °C/No O_3	1	Start-up/20 days	35	0.92	3.46	519	0.07
			70	0.80	2.64	443	0.04
	2	15	105	0.89	3.31	509	0.06
			140	0.83	2.94	440	0.05
	3	10	175	0.79	2.44	461	0.04
			210	0.88	3.12	474	0.06
			245	0.87	2.97	388	0.06
	4	20	281	0.93	3.39	367	0.09
			316	0.91	3.32	372	0.08
	5	15	350	0.90	3.01	337	0.07
			385	0.89	3.11	354	0.08

Table S5.7 Alpha diversity for archaeal populations in the four anaerobic digesters.

Reactor	Period	SRT (days)	Days	Diversity Indices			
				Simpson	Shannon	Chao-1	Evenness
20 °C/ WAS O_3	1	Start-up/20 days	35	0.59	1.28	22	0.17
			70	0.58	1.27	21	0.17
	2	15	105	0.63	1.32	21	0.18
			140	0.53	1.14	21	0.15
			175	0.51	1.07	20	0.15
	3	10	210	0.68	1.35	17	0.23
			245	0.66	1.35	17	0.23
	4	20	281	0.61	1.25	18	0.19
			316	0.59	1.12	15	0.2
	5	>15	350	0.7	1.43	19	0.22
			385	0.58	1.28	24	0.17
20 °C/ AD O_3	1	Start-up/20 days	35	0.62	1.43	26	0.17
			70	0.65	1.42	22	0.19
	2	15	105	0.59	1.24	26	0.15
			140	0.61	1.25	26	0.15
			175	0.67	1.5	23	0.19
	3	10	210	0.7	1.65	24	0.22
			245	0.68	1.49	18	0.25
	4	20	281	0.34	0.8	22	0.11
			316	0.43	0.9	20	0.13
	5	>15	350	0.48	0.99	17	0.16
			385	0.59	1.17	22	0.16
35 °C/ WAS O_3	1	Start-up/20 days	35	0.71	1.56	28	0.19
			70	0.57	1.29	26	0.16
	2	15	105	0.66	1.38	20	0.2
			140	0.62	1.33	22	0.18
			175	0.7	1.45	26	0.19
	3	10	210	0.76	1.66	28	0.21
			245	0.77	1.67	29	0.23
	4	20	281	0.43	1.04	17	0.17
			316	0.52	1.11	21	0.16
	5	>15	350	0.61	1.24	18	0.2
			385	0.54	1.18	18	0.22
35 °C/No O_3	1	Start-up/20 days	35	0.63	1.36	25	0.16
			70	0.54	1.16	24	0.13
	2	15	105	0.61	1.35	26	0.15
			140	0.68	1.38	18	0.22
			175	0.67	1.56	32	0.18
	3	10	210	0.57	1.06	17	0.17
			245	0.63	1.21	17	0.2
	4	20	281	0.59	1.11	18	0.18
			316	0.16	0.43	16	0.1
	5	15	350	0.66	1.37	16	0.24
			385	0.34	0.71	18	0.14

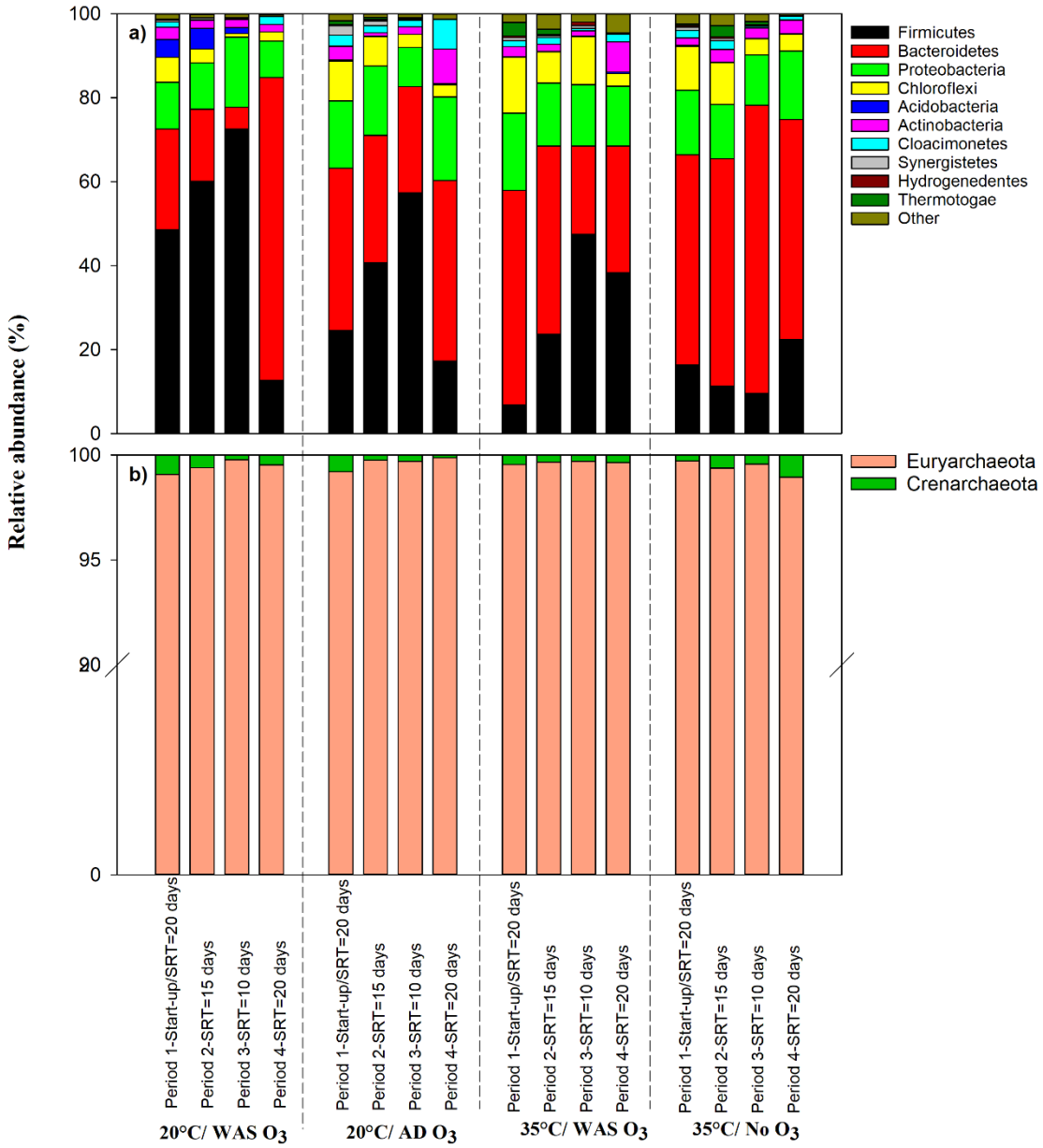

Fig. S5.6. Relative abundance of phylogenetic groups at phylum level in the anaerobic digesters operated at different conditions (20 °C vs 35 °C; SRT of 10, 15, and 20 days; and ozonated vs non-ozonated feed) for bacteria (a) and archaea (b). Each phylum is coded with a unique pattern, and the height of each bar represents their respective relative abundance. Phylogenetic groups accounting for ≤2% of all classified sequences are summarized in the artificial group "others". In panel (b) the break range for the Y axis is 20-90%, with 5% intervals after the break. Each bar represents averages of the following days: SRT = 15 days: Day 105, 140 and 175, SRT = 10 days: Day 210 and 245, and SRT =20 days: Day 281 and 316.

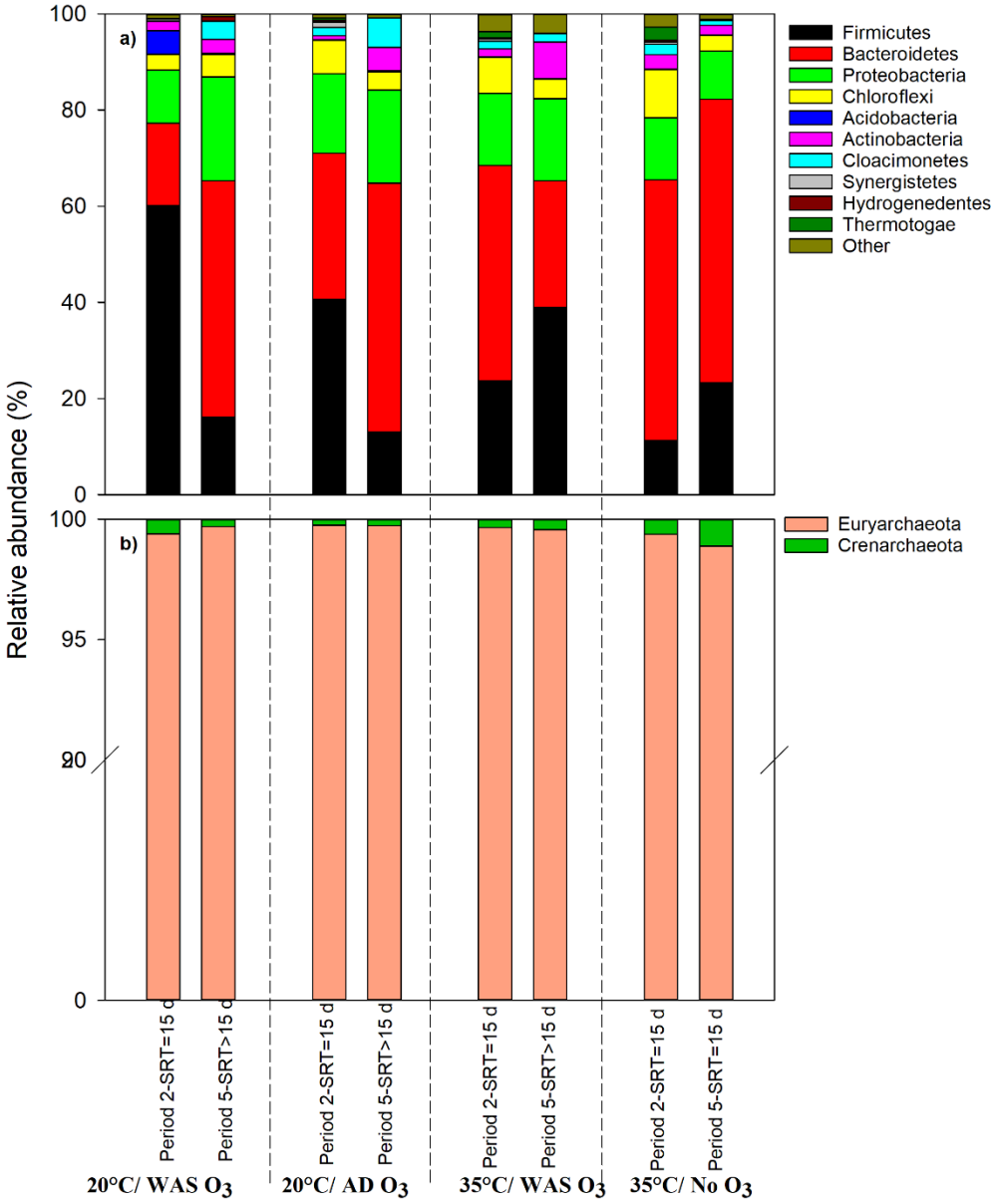

Fig. S5.7. Relative abundance of major phylogenetic groups at phylum level detected in the anaerobic digesters under coupled or decoupled SRT-HRT conditions (Period 3-SRT=15 days, Period 5-SRT>15 days). Bacterial phyla are presented in panel (a) and the archaeal phyla are presented in panel (b). Phylogenetic groups accounting for ≤2% of all classified sequences are summarized in the artificial group "Others". In panel (b) the break range for the Y axis is 20-90%, with 5% intervals after the break. Note: The SRT for the 35 °C/ WAS O_3, 35 °C/ No O_3, 20 °C/ WAS O_3, and 20 °C/ AD O_3 reactors in Period 5 was 24, 15, 19, and 21 days, respectively. Each bar represents average of the following days: SRT = 15 days: Day 105, 140 and 175, and SRT >15 days (Period 5): Day 350 and 385.

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CHAPTER 6

Plant-wide modeling of anaerobic digestion combining sludge ozonation at low mesophilic temperature: Exploring energy, carbon footprint and cost benefits

Connecting text: In chapters 3 and 5, the feasibility of high rate anaerobic digestion at low mesophilic temperature, combining ozone pre-treatment of biosolids, was demonstrated by operating lab scale reactors. However, in full-scale water resource recovery facilities, important questions that remain concern the differences in energy consumption, GHG emissions, and carbon footprint, due to ammonia and COD flow variations induced by anaerobic digestion. The findings from chapters 3 and 5 were used to construct a plant-wide model, to evaluate direct and indirect emissions in terms of CO_2 equivalents per day. We identified GHG emissions from every step of the life cycle of the biosolids based on a "cradle-to-grave" analysis, from the start of the treatment train to disposal at landfill or land application for agriculture. Integrating sludge ozonation was found to impart added benefits to AD including feasibility at low mesophilic temperature (20 ºC), enhanced digester performance in terms of VSS reduction and biogas production, higher energy sustainability, and reduced carbon footprint and operational cost, as compared to conventional AD at 35 ºC.

The results of this research will be submitted to the journal of *Journal of Bioresource Technology* by the end of 2020 under the title "Plant-wide modeling of anaerobic digestion combining sludge ozonation at low mesophilic temperature: Exploring energy, carbon footprint and cost benefits". The authors will be listed as Zeinab Bakhshi, Shameem Jauffur, and Dominic Frigon.

6.1 INTRODUCTION

The application of low mesophilic temperature (20 ºC or below) in anaerobic digestion (AD) technology holds economic and environmental incentives over mesophilic (30-42 ºC) and thermophilic (50-65 ºC) approaches. Low operational temperatures can effectively improve the energy balance of AD and represent an interesting option in northern countries, where temperatures are much lower than 35-37 ºC (Bialek et al., 2013). According to Grant and Lin (1995), depending on the temperature of the matrix to be treated and the climatic conditions, it is not always practical to operate at the optimum temperature range because of the higher energy requirements. The advantages of using high mesophilic and thermophilic temperatures are faster reaction rates and smaller digester footprints, but the disadvantages involve a greater energy cost and the risk of losing treatment capacity due to a failure in the reactor heating system (Rittmann & McCarty, 2001a).

Sub-optimal temperature AD has been successfully applied to treat a wide range of wastewaters (Connaughton et al., 2006; Enright et al., 2009; McKeown et al., 2009) and wastes such as manure (Alvarez & Lidén, 2009). However, AD of waste activated sludge (WAS) is comparatively challenging (Clarke, 2018). The high solids content, large particle size, variability, high concentration of recalcitrant matter and heterogeneous nature of the composition makes it difficult to control the process (Fan et al., 2018). Some authors argue that high temperature mesophilic digestion (35-37 ºC) is not very efficient at reducing biosolids with significant levels of particulate matter, and at deactivating pathogenic microorganisms (Song et al., 2004). This problem is mainly seen when digesting WAS from an activated sludge system with a high solids retention time (SRT) because WAS becomes less biodegradable with increasing SRT (Ekama et al., 2007). Other authors advocate the application of thermophilic digestion (50-65 °C) as a better option to improve the efficiency of WAS AD in terms of methane yield and volatile solids reduction (Bougrier et al., 2008; Ennouri et al., 2016; Gavala et al., 2003). In both high mesophilic and thermophilic digestion, the heat energy requirement is significant to sustain the anaerobic digestion process (Tchobanoglous & Burton, 1991). Hence, despite an enhanced treatment, high mesophilic and thermophilic AD can be detrimental to the carbon footprints and energy recovery of water resource recovery facilities (WRRFs).

Environmental models have shown that WAS treatment and disposal contribute to the emission of greenhouse gases (GHG) in the atmosphere, as also highlighted by the Intergovernmental Panel on Climate Change (IPCC) Guidelines used for compiling GHG inventories of member countries (IPCC, 2006). Direct emission of methane (CH_4) and nitrous oxide (N_2O) resulting from biological processes can occur from sewers and during wastewater and WAS treatment. Indirect GHG emissions occur from the consumption of electricity, burning of fossil fuels for transportation, use of chemicals for sludge dewatering and disposal of biosolids at landfills (Parravicini et al., 2016). Hence, optimizing energy efficiency and reducing carbon footprint of treatment infrastructures are fundamental to enhance the sustainability of AD technologies. These enhancements can also improve the economic feasibility since AD of wastes is highly dependent on economic incentives from the government to be sustainable (Vasco-Correa et al., 2018). The collection and processing of waste can be more costly than the value of the end product (biogas and digestate) (Appels et al., 2008). According to Zaks et al. (2011), even with a 5-fold growth in AD technology from 2000 to 2010, many roadblocks need to be removed to realize the climate, air, water, and development benefits that would foster widespread adoption. On this basis, high mesophilic and thermophilic AD can be challenging since the energy balance between inputs (pretreatment, AD process, upgrading process, collection and transportation) and output energy (potential energy content of the waste) has yet to be improved.

Recently, the feasibility of performing AD of WAS at low mesophilic temperature (20 ºC) combining solids pre-ozonation has been demonstrated (Chapter 3 and 5). Operation of laboratory-scale reactors at 20 ºC with pre-ozonated WAS achieved higher volatile suspended solids (VSS) destruction and methane yield than conventional AD at 35 ºC with untreated WAS (+35% and +14%, respectively). Hence, the problem associated with the rate-limiting step of hydrolysis can be circumvented by pretreating the complex organic matter with ozone. Implementation of such a hybrid system in a 2,000 mL laboratory scale AD led to a higher performance at 20 °C with an enhanced VSS reduction by 20% and biogas production by 29% as compared to conventional anaerobic digestion at 35 °C with untreated sludge. Ozonating the anaerobic digestate rather than the WAS further increased the VSS reduction and biogas production by almost 10% showing that the point of ozonation is an important factor to consider when implementing low temperature anaerobic digestion (Chapter 5). A simple energy balance analysis has shown that such combined AD-ozonation process operated at low mesophilic temperature (20 ºC) can result in 35% higher

net energy production by the AD (net energy balance: +174 GJ/d) than a traditional AD at 35-37 ºC (net energy balance: +129 GJ/d), thus offering a more energetically sustainable option (Chapter 3). However, operation of full-scale AD at 20 ºC coupled with sludge ozonation would result in in a return flow to the activated sludge system of higher COD and ammonia levels, which in turn could increase energy consumption in the aeration basin. Thus, information on plant-wide changes in energy, carbon footprints and operational costs from the installation of such a process are required. Understanding the performances of the AD-ozonation process to improve sustainability is crucial for its implementation at full-scale.

The key aspects were investigated in the current study by first calibrating a plant-wide treatment process model (including activated sludge and anaerobic digestion processes) using routine measurements obtained from a full-scale WRRF near Montreal (Quebec, Canada), and then using the simulation results to evaluate the plant-wide energy consumption and greenhouse gas (GHG) emissions in terms of CO_2 equivalent. Specifically, the objectives were four-fold: (i) to quantify the impacts of temperature and sludge pretreatment on key operating variables (VSS destruction, biogas, and ammonia) and energy benefits of various configurations, (ii) to determine the carbon footprint associated with each simulated configuration (iii) to perform a cost benefit analysis to determine associated expenditures with each configuration, and (iv) to identify the most sustainable operational strategies with the lowest energy and carbon footprint without compromising treatment quality. It has been suggested that the overall energy consumption of many WRRFs could be reduced by 10-40% through operational improvements (Aymerich et al., 2015; WEF, 2009). This current approach effectively helps to identify sustainable alternatives to traditional treatment strategies with emphasis on sustainability, energy consumption and GHG emissions.

6.2 MATERIALS AND METHODS

For developing the plant-wide treatment process model, flow and wastewater quality measurements from routine sampling and analysis were provided by the Régie d'Assainissement des Eaux du Bassin de LaPrairie (RAEBL) WRRF (Montréal, Canada). These data were used for testing and validating the developed model.

6.2.1 Description of WRRF under study

The modeled full-scale treatment facility was the RAEBL WRRF located on the south shore of Montréal and collecting wastewater from five (5) municipalities, namely LaPrairie, Candiac, Delson, Sainte-Catherine, and Saint-Constant. The treatment plant used a biological activated sludge process to remove carbonaceous and nitrogenous biochemical oxygen demand (BOD) from a stream of combined, domestic and industrial, wastewaters. The average wastewater flow rate in 2016-2017 was 55,543 m^3/d. The treatment facility included conventional primary treatment (screening and degritting), followed by four parallel rectangular aerated bioreactors (8,400 m^3 each), and completed by three mixed liquor suspended solids (MLSS) rectangular clarifiers (6,127 m^3 each). The clarified effluent is disinfected by ozonation and, ultimately, discharged into the St-Lawrence River. A fraction of the settled solids was returned to the bioreactors as recirculated activated sludge (RAS). WAS is thickened using two dissolved air flotation (DAF) units, and subsequently fed into an anaerobic digester system consisting of a thermophilic hydrolyzer unit (730 m^3, 55 ºC, SRT of 48 hours) and two upper-temperature mesophilic anaerobic digesters (3,100 m^3 each, 35-37 ºC, SRT of 19 days). Digested sludge was mechanically dewatered in centrifuges to produce a sludge cake with up to 20% dryness. Biogas resulting from the digestion process is drawn from the headspace and transferred to generator engines to produce heat to further dry the biosolids to 90%, before being transported to landfill, mining and agricultural sites.

6.2.2 Plant-wide model development and calibration

6.2.2.1 Plant configuration

The existing process flow diagram of the treatment facility was used to construct a plant-wide model configuration (Fig. 6.1). Both aerobic and anaerobic processes were modeled based on stoichiometry and biokinetics, described by the integrated Activated Sludge Anaerobic Digestion Models (ASDM) included in the BioWin Simulation Package (v.5.2.0.1162). The ASDM contains 82 processes and 46 state variables (Elawwad et al., 2019). The model was built using a plug-flow design with four compartments. The settling tanks were modeled as a single non-reactive settler based on the Vesilind Settling Model with five layers (Takács et al., 1991), with the wastewater channeled to the 3rd layer, the clarified effluent leaving from the top layer, and the settled biomass leaving from the base to be recycled back to the bioreactor as RAS. Sludge thickening by the DAF and dewatering units were assumed to be ideal (with a constant split fraction and characteristics),

with no hold-up volume (Jeppsson et al., 2007). The anaerobic system was modeled as one unit; this approach was justified considering that the sludge was split into two parallel digesters.

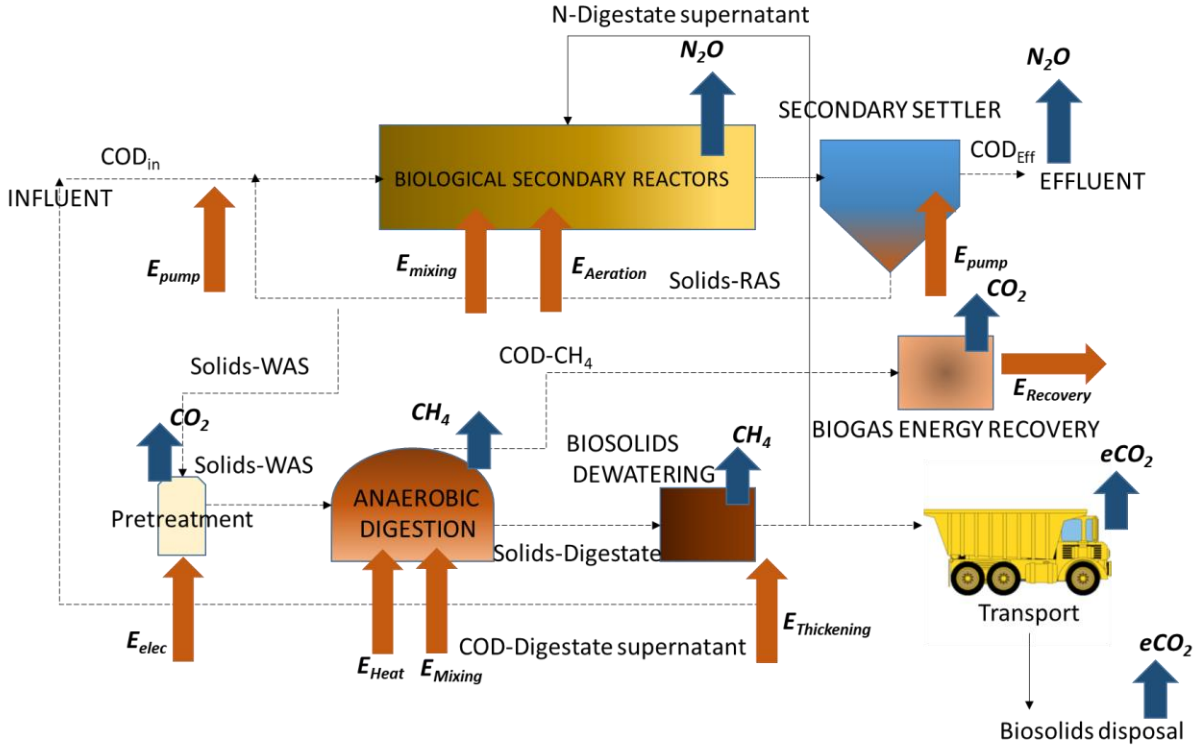

Fig 6.1. Schematic of the RAEBL WRRF, with the main wastewater treatment stream (top), the anaerobic digestion and sludge handling stream (bottom), and potential GHG Emission at different treatment stages. In the wastewater stream, the bioreactors HRT and SRT were 15hr and 7 days, respectively. In the sludge stream, the hydrolyzer was operated at 55 ºC, with an SRT of 48 h, and the digester was maintained at 35-37 ºC, with an SRT of 19 days. Detailed descriptions are presented in the text.

6.2.2.2 Model calibration and evaluation

Model parameters for ASDM were kept at default values, except for the influent characteristics. Since mathematical calibration methods are used for activated sludge or anaerobic digestion models, and none exists for plant-wide models (Batstone et al., 2002; Rieger et al., 2012; Vanrolleghem et al., 2003), a step-wise calibration procedure, inspired by Kazadi Mbamba et al. (2016), was adopted. By adjusting a few kinetic parameters at a time, discrepancies between the model output and static measured dataset were reduced.

Step 1-Calibration of Activated Sludge Model: The model was calibrated using one-year operational data from the RAEBL WRRF before the installation of the anaerobic digesters (Jan 2016-Dec 2016). The idea was to fit key operational variables, such as MLVSS, effluent NH_4^+-N and NO_3^--N, to match their corresponding measurements by adjusting the influent COD fractions while keeping the stoichiometric and kinetic parameters at their default values. The different influent COD fractions used in the model are shown in Table S6.1. The actual variations in influent loads, temperature, and plant operational conditions were used as inputs for the dynamic simulations. The aeration system setup was reproduced in the model and comprised of moderate bubble diffusers (2 ramps with 20 diffusers per basin). Since unintended anoxic zones leading to significant denitrification were present in the aeration basins, the oxygen transfer from the air supplied by the blowers was calibrated by adjusting the dissolved oxygen (DO) in the first three aeration basins. To capture the effluent NO_3^--N concentration, the DO level in the first three aeration tanks was adjusted at 1/3 of the DO, measured at the end of the parallel plug-flow aeration tanks; the actual measured DO was used for the final aeration basin.

Step 2- Calibration of Anaerobic Digestion Model: The anaerobic processes of the treatment facility were calibrated using the first six months of historical data after the installation of the anaerobic digester units (July-Dec 2017). Predictions of key parameters, such as biogas production, digestate VSS, and digestate NH_4^+-N, were fitted to the measured data by adjusting the hydrolysis rate.

Step 3-Calibration of ozone pretreatment: Since no full-scale facility data was available for anaerobic digestion combining biosolids ozonation, the ability of the model to capture this process was developed and calibrated using lab-scale anaerobic sequencing batch reactors (ASBRs) data described in chapter 5. Data from the following three reactors were considered: anaerobic digesters operated at 20 °C and fed with ozonated WAS (20 °C/O_3 WAS), 35 °C and fed with ozonated WAS (35 °C/O_3 WAS), and 35 °C fed with non-ozonated WAS (35 °C/No O_3 WAS). The model was calibrated using operational data gathered over a three-month period. Since BioWin v.5.2.0. does not have an inbuilt module to model oxidative processes, such as ozonation, the Thermal Hydrolysis module was calibrated to account for the measured ozonation transformation stoichiometries, including solubilization of particulate matter and increased degradability of recalcitrant COD fractions. Biomass fractions, model parameters and kinetic coefficients for

ozonation of WAS were initially adopted from Isazadeh et al. (2014), and slightly adjusted to fit key variables, such as biogas production, digestate VSS, and NH_4^+-N, to the measured data (Table S6.1). The model proposed by Isazadeh et al. (2014) provides a comprehensive and precise approach to describe the action of ozone on biosolids, extending the activated sludge model ASM3 by explicitly separating it into two distinct ozone processes of biomass inactivation and non-biomass transformation.

To evaluate the quality of the calibration, the goodness-of-fit of the dynamic simulations was assessed by Major Axis Regression (MAR) (Model II regression) on the measured and predicted concentrations (Mesplé et al., 1996). Biases were evaluated by assessing the slopes (should be 1 for no bias) and intercepts (should be 0 for no bias) of resulting simulated-measured fits. Finally, the R^2 was calculated as a final quality measure.

6.2.3 Modeling impacts on energy and carbon footprint

A modeling scenario analysis was performed to compare the performances of the RAEBL treatment facility in terms of energy balances, GHG emission, carbon footprint, and cost analysis, upon implementation of various AD configuration, including a system at low mesophilic temperature (20 ºC) with ozonated sludge.

6.2.3.1 Model configurations

Six operational configurations were analyzed using the calibrated model formulated in Biowin (v.5.2.0). The base case scenario was the one already implemented at the RAEBL WRRF, and comprised of activated sludge treatment and an AD system with a hydrolyzer at 55 °C and two anaerobic digesters at 35 °C. The other scenarios consisted of the same activated sludge system, but different operational conditions for the anaerobic digesters as summarized in Table 6.1. The different scenarios were analyzed based on 150 days of simulation with dynamic influent conditions (July-Dec 2017). The outcomes of each scenario were compared in terms of biogas production, VSS destruction, and digestate NH_4^+-N level.

Table 6.1. Description of the 6 modeling scenarios analyzed for RAEBL full-scale treatment facility with different operational AD configurations.

Scenario	Pretreatment		Methanogenic Digester Temperature	Notation
	Hydrolyzer at 55 °C	WAS ozonation		
1[a]	Yes	No	35 °C	35 °C/ WAS HD[b]
2	No	No	35 °C	35 °C/ No HD & No O_3
3	No	Yes	35 °C	35 °C/ WAS O_3
4	Yes	Yes	35 °C	35 °C/ WAS HD & O_3
5	No	No	20 °C	20 °C/ No HD & No O_3
6	No	Yes	20 °C	20 °C/ WAS O_3

[a] Existing full-scale base scenario; [b] HD: Hydrolyzer

6.2.3.2 Impact on plant energy efficiency

The energy requirement for the scenarios described above was determined by calculating the energy consumed for operating the activated sludge system (pumps, blowers, mixers…etc.), biogas plant, and digestate processing and handling units. The equipment and installations considered for the energy computation are listed in Table S6.2. Net energy production was determined as useful energy derived from biogas and heat recovery minus energy input to the system. For the base case scenario (hydrolyzer at 55 °C and AD at 35 ºC), the average monthly energy consumption (kWh) for the different components of the aerobic treatment system was obtained directly from monthly bills for electricity supplied by Hydro Quebec national grid during the period July to December 2017. For the other scenarios, the outputs of the plant-wide model, described in Section 6.2.2, were used to estimate the energy consumption. The thermal energy consumption and heat recovery from the biogas production were determined using equations listed in Table S6.3. These included energy required to heat up the digester or hydrolyzer (E_{req}), energy produced from biogas (E_{CH4}) and estimated from the actual methane production (V_{CH4}) (Puchajda & Oleszkiewicz, 2008), heat recovery (E_{rec}) resulting from a heat exchanger used to capture heat energy from sludge leaving the digester (Puchajda & Oleszkiewicz, 2008), and energy required for sludge ozonation based on the amount of ozone dosed per gram of solids (E_{O3}). Any difference between the operational and ambient air temperature resulted in heat loss (E_{loss}). The heat loss (E_{loss}) was determined by the product of the areas of the digester or hydrolyzer chamber (i.e. the side walls, floor, and cover) and the difference in temperature of the digester (T_{dig}) and ambient temperature (T_{air}) and their respective heat transfer coefficients (h_i) based on Tchobanoglous and Burton (1991) (Table S6.3, Eq. S6.2). The net energy production (E_{net}) was computed based on the difference between the

energy produced (E_{CH4} and E_{rec}) and energy expended (E_{req}, E_{loss}, E_{mix} and E_{O3}) during the AD process.

For estimating the heat requirement, the following assumptions were made: an average WAS flow of 327 m^3/d (during period July-Dec2017) to the hydrolyzer and/or digester, an average temperature of incoming sludge (T_{in}) for winter, spring, summer and fall of 12.5 °C, 17 °C, 20 °C, and 17 °C, respectively (based on operational dataset from Plant Operator), a hydrolyzer temperature of 55 °C and a digester temperature of 20 or 35 °C ($T_{digester}$). To determine heat loss from the system, the heat transfer coefficients for the walls, and cover of the tanks were assumed to be 0.23 W/m^2 °C, and 0.38 and 0.57 W/m^2 °C for the floors of the hydrolyzer and anaerobic digesters, respectively (Tchobanoglous & Burton, 1991). The average ambient air temperatures (T_{air}) for winter, spring, summer and fall were -15 °C, 12 °C, 25 °C and 12 °C, respectively (EnvironmentCanada, 2017). The temperature of the ground (T_{ground}) was assumed to be -3 °C, 5 °C, 15 °C and 5 °C for winter, spring, summer and fall, respectively (EnvironmentCanada, 2010). Recoverable energy is comprised of energy produced from biogas and heat recovered from heat exchangers. The biogas energy was estimated based on the average data obtained from the plant-wide model. Ozone production for sludge ozonation required electrical energy at 12.5 kWh/ kg O_3 (Chu et al., 2009).

6.2.3.3 Impact on plant carbon footprint analyses

The carbon footprint of the RAEBL WRRF, under each operational scenario described in Section 6.2.3.1, was determined to assess possible impacts on the environment. Although production of energy from biomass represents an interesting avenue for waste treatment, biogas production can generate significant impacts to the environment in terms of GHGs (Appels et al., 2011; Hoppe & Sanders, 2014). Direct and indirect GHG emissions were inventoried with the balance boundary set to include the aerobic and anaerobic systems, and sludge treatment, handling and transport to disposal sites. The carbon emission for each scenario was segregated into direct and indirect emissions. Direct emissions included sources such as the biogenic carbon fractions of the digestate, CH_4 flaring, and heat and electricity generation from CH_4. Direct GHG emissions were determined based on their mass transfer coefficients (d^{-1}), dissolved gas equilibrium concentrations (g/L), reactor volume, and global warming potentials (GWPs). Indirect emissions included operations consuming energy (pumping and mixing). This was derived using time-dependent electricity

consumption (kWh/d), and the GWPs of the emitted GHGs. Indirect emissions resulted from the use of materials and chemicals. These were estimated using time-dependent material consumption (kg/d) and the CO_2 factor associated with the materials being used. Finally, emission from sludge transport, also considered as an indirect emission, was calculated based on one ton of sludge transported on one-kilometer (t.km) basis. Emission factors (EFs) from the IPCC Guidelines (Eggleston et al., 2006) were used to estimate the gas emissions and carbon footprints associated with each scenario. After treatment, the sludge was transported to landfill sites (Saint-Nicéphore and Sainte-Sophie, QC), a mining site for use as landscaping material (Black Lake, QC) and agricultural lands in Godmanchester, Mirabel, and Ryan Noyan (QC) for use as fertilizer/soil conditioner. The amount of sludge transported to these sites, and their respective distance (in Km) from the RAEBL plant, are provided in Table S6.4.

6.2.3.4 Impact on plant direct GHG emissions

Emission of N_2O and CH_4 can occur at different treatment stages at the RAEBL facility (Fig. 6.1). The potential sources of N_2O were identified as coming from the bioreactors through stripping from processes of nitrification/denitrification and effluents. Direct CH_4 emission was identified as stemming from the sludge holding tanks, hydrolyzer unit, anaerobic digesters, and sludge dewatering system. These possible sources of N_2O and CH_4 are supported in the published literature (Corominas et al., 2012; Kampschreur et al., 2009). Emission of CO_2 was mainly accounted for by the transport of treated sludge to the landfills, mining sites, and agricultural lands. The amount of N_2O and CH_4 emitted into the atmosphere from the liquid and gas phase of the treatment chain under the different analyzed scenarios was estimated using BioWin (v 5.2.0). The ASDM model in BioWin has been extended to include N_2O and CH_4 production during aerobic and anaerobic treatment. The N_2O EF, expressed as kg N_2O-N emitted per influent kg TN, varied between 0.601-0.870% for the different scenarios. These values were in the medium to high range due to denitrification occurring in the aeration basin, compared to other full-scale WRRFs, typically between 0.001-0.65 kgN_2O/kgN (%), which vary widely depending on a plant's configuration or operation (Filali et al., 2013; Law et al., 2012). The calculation of the climate impact of the N_2O and CH_4 emitted was performed by considering the GWP of 298 kg CO_2e/ kg N_2O and 25 kg CO_2e/kg CH_4, respectively, for a timeframe of 100 years (IPCC, 2006). CH_4 emission, associated with storage, was estimated using a factor of 0.12 kg CH_4/kg BOD_5 (CCME, 2009).

6.2.3.5 Impact on plant indirect GHG emissions

Indirect emissions are due to energy consumption (electricity) to operate each process. A GHG EF of 0.856 kg CO_2e/kWh was assumed for electricity use in Quebec (NRC, 2018b). Emission from sludge transport, also considered as an indirect emission, was calculated based on one ton of sludge transported on one-kilometer (t.km) basis. The truck's EF was calculated by considering an average truck fuel consumption of 7 miles per gallon, following an industry standard range of 6-7 mpg (NRC, 2000). In the Canadian National Railway Company GHG calculator, for an average truck weight of 14.5-16 tons, an EF of 63.8 g CO_2e/ton-km was used (CNRC, 2016). Indirect emissions can also occur through chemical usage such as ozone production using compressed oxygen and sludge dewatering through the addition of chemical polymers (usually 5 kg per ton of dry solids) (Tchobanoglous et al., 2013). Furthermore, an EF of 0.41 kgCO_2e/kgO_2 (NRC, 2017) and 2.62 kgCO_2e/ kg of sludge (Parravicini et al., 2016) was used to compute emissions resulting from ozonation and polymer-based sludge dewatering, respectively. The EFs for the final disposal of sludge by landfilling and land application, used in the current study, were 0.13 kg CH_4/ tons of dry sludge and 0.2875 kg N_2O/ tons of dry sludge, respectively (Doka, 2003). About 70% of the treatment sludge from the RAEBL treatment plant are disposed at landfills and 30% are used for land applications. All sources of direct and indirect GHG emissions are quantified and their respective EFs are shown in Table S6.5.

6.2.3.6 Impact on plant cost benefits

The installation of AD systems represents a major capital investment and requires careful engineering and economic considerations. In this context, a comprehensive cost analysis was performed to determine the economic sustainability of the different operational configurations described in Section 6.2.3.1. The main aim was to assess the economic performance of an installation combining AD and sludge ozonation at low mesophilic temperature (20 ºC), as compared to conventional standalone biogas system, operated at 35-37 ºC. Cost-benefit calculations were made based on well-defined input parameters from literature and experimental results. For developing the economic model, the cost of electricity was estimated based on the rates for industrial consumptions of 3.43 ¢/kWh from HydroQuebec (2018). The consumption of diesel was estimated using an average fuel consumption rate of 7 miles per gallon (NRC, 2000) and an average diesel price of $1.30 (NRC, 2016). The cost of one kilogram of chemical polymer used for sludge dewatering was obtained from the RAEBL database at a rate of 4.29 $/yr. Average

round-trip distances for transport of treated biosolids at the disposal sites (Table S6.5) were obtained from the Subcontractor VIRIDIS Environnement, assigned to carting away the sludge from the RAEBL facility. While factors such as traffic conditions, weather, fleet composition, and transport infrastructure can have an impact on the accuracy of the average vehicle fuel consumption and subsequent emission estimates, they represent minor factors on the global economic cost (Reyna et al., 2015) and were, therefore, excluded from current assessments. The disposal cost of sludge was estimated at $100/tones of sludge (LeBlanc et al., 2009; RECYC-QUÉBEC, 2018).

6.2.3.7 Conventional vs proposed biogas system configuration

To further the comparison of a conventional biogas installation operated at 35-37 ºC using non-ozonated feedstock with a system combining AD with sludge ozonation at low mesophilic temperature (20 ºC), a performance and carbon footprint assessment was performed on a typical WRRF, to provide plant operators and decision makers/investors with a quick look at the potential benefits that may be derived from the new proposed configuration. A virtual treatment system was designed using Biowin (v 5.2.0) with the COD fractionations set at default values and receiving an average wastewater flow of 60,000 m^3/d and influent COD of 600 mg/L. The stochiometric and biokinetic parameters were also set at BioWin default values. The DO in the aeration basin was adjusted to 2.0 mg/L (full aeration). The temperature of the aeration basin was set at 20 °C for summer and 12 °C for winter. The SRT of the anaerobic digesters was adjusted to 20 days. Simulations were performed to assess the net energy, GHG emission, and carbon footprint associated with the operation of the typical WRRF with a biogas plant at 35 °C using raw sludge and an anaerobic digester at 20 °C with sludge ozonation. The simulation outcomes of the two systems were compared and used as a quick reference tool to provide stakeholders with more insights on the new proposed biogas system configuration relative to the already existing AD practices.

6.3 RESULTS

6.3.1 Calibration

The RAEBL WRRF anaerobic digesters were installed in July 2017. Thus, two periods of operation were used to calibrate the plant-wide model. First, the one-year period of January-

December 2016 (i.e., before the installation of the anaerobic digesters) was used to adjust the influent COD fractions and the activated sludge aeration efficiency along the length of the reactor, in order to fit the concentrations of MLVSS, effluent NH_4^+-N, and NO_3^--N. The simulated parameters, after calibration, were in agreement with the corresponding measured data, showing an R^2 above 0.75, with slightly significant biases, but generally less than 7% (Fig. S6.1, Table S6.6). Second, the 150-day period of July-December 2017, after the installation of the anaerobic digester, was used to adjust the hydrolysis rate by fitting the digester biogas production rates, and concentrations of VSS and NH_4^+-N, while making sure that the initially simulated results of the fitted operation data remained satisfactorily predicted (Figs. S6.2 and S6.3). Again, a good fit was achieved with minimal relative biases (Table S6.7).

To implement and calibrate the pre-ozonation transformation in the BioWin model, it was resolved to use the thermal hydrolysis module of BioWin. The thermal hydrolysis unit in BioWin consisted of set of stoichiometric transformations, proportional to the ozone dose. At the base, we used the stoichiometry description of the ozone transformation, proposed as an extension to AMS3 by Isazadeh et al. (2014); the stoichiometric values obtained by Isazadeh et al. (2014) were utilized with slight adjustments to optimize the fit and minimize the biases of the anaerobic digestate VSS, biogas flow rate, and digestate NH_4^+-N (Fig. S6.4; Table S6.8).

An additional scenario involving the operation of an ASBR at 20 ºC with non-ozonated WAS (20 °C/ No O_3) was also investigated. However, the operational data of laboratory scale ASBR were used for model calibration (Chapter 3). The scenario provided some insights of the model's response under such scenario, where the biogas production rate was found to be minimal (average of 3.1 mL/h).

6.3.2 Plant-wide response to various WRRF configurations

Once calibrated, the plant-wide operation responses to the scenarios listed in Table 6.1 were simulated using the base plant data from July 2017 to December 2017 (Fig. 6.2 and Fig. S6.5). The bar charts indicate the percentage of (average simulation of each scenario / average measurement of the base case scenario)-100. The base case scenario involved digester operation at 35 °C with thermophilic hydrolysis at 55 °C (35 °C/ WAS HD). Average values of measured data for the base case scenario were VSS destruction: 41±2%; Biogas flowrate: 105±1 m^3/h and NH_4^+: 1423±16 mg-N/L. A slight average divergence was observed for the simulated data over the measurements

for the base case scenario (35 °C/ WAS HD) corresponding to the current configuration of the RAEBL WRRF; nonetheless, the average simulated data are acceptably close to the plant operation results. Analysing the configuration variants, the highest VSS destruction (average of 58.8%) and biogas production (115.5 m^3/h) were obtained for digester operation at 35 °C, with WAS ozonation followed by thermophilic hydrolysis at 55 ºC as pretreatment (35 °C/ WAS HD & O_3) (Fig. 6.2a, b and Fig. S6.5 a, b). However, simulations without thermophilic hydrolysis (35 °C/ WAS O_3) indicate only a slight reduction in the performance, suggesting that WAS pre-ozonation could be more beneficial for the RAEBL than thermophilic hydrolysis as WAS pretreatment. Interestingly, operation of the digester at 20 ºC with WAS ozonation (20 °C/ WAS O_3) resulted in a VSS destruction and biogas production of almost 14% and 9%, respectively, higher than the base case (35 °C/ WAS HD) implemented at the RAEBL WRRF. This plant configuration was also more efficient, in terms of VSS reduction and biogas production, than simply operating the anaerobic digester at 35 °C without any pretreatment (35 °C/ No HD & No O_3). Hence, pretreatment by WAS ozonation appears to be more effective at enhancing digestion than thermophilic hydrolysis.

As part of the simulation of the six plant configurations, the NH_4^+-N concentrations in the digestate were predicted. The concentrations correlated with the VSS destruction with the digesters receiving pretreated WAS showing higher NH_4^+-N levels as compared to the digesters with no pretreatment (Fig. 6.2c and Fig S6.5c). This recirculation to the main aeration basin of NH_4 -N in the centrate from the centrifuge dewatering process suggests possible negative consequences for the energy and carbon footprint of the plant due to enhancing anaerobic digestion. These questions were investigated by simulating plant-wide energy consumption and carbon emission are described in subsequent sections.

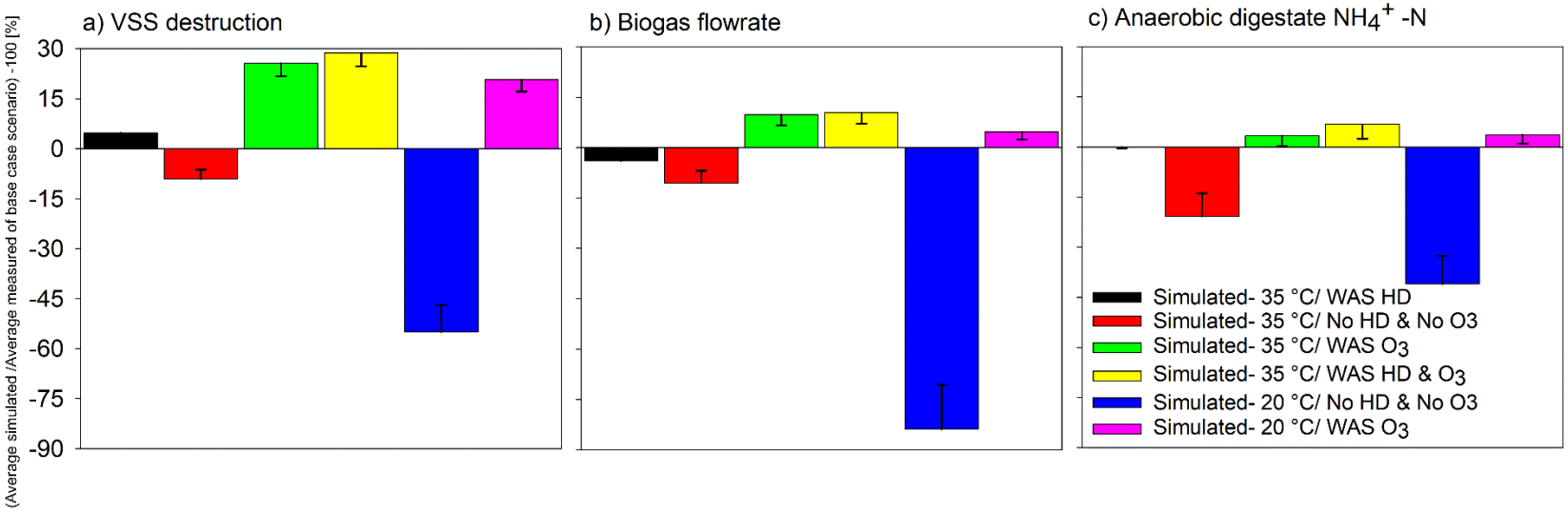

Fig 6.2. Scenario analysis of anaerobic digesters at different temperatures (20 °C and 35 °C) and pretreatments (thermal hydrolysis [HD] and WAS ozonation [O_3]). A) VSS destruction (%); b) Biogas flowrate (%); c) Anaerobic digestate NH_4^+-N (%). The error bars indicate the standard error measured at steady state.

6.3.3 Plant-wide Energy performance

In chapter 3 we demonstrated that operating the digester at 20 ºC instead of 35 ºC, while pre-ozonating the WAS, could increase the net energy recovery by 35%, when considering the ambient temperature to be 10 ºC. However, the higher aeration needs for nitrification of the additional NH_4^+-N recirculated from the digester could affect the overall plant energy performance. In order to investigate this possibility, we used the plant-wide model to quantify the energy budget of the six operational configurations (Fig. 6.3). The plant-wide power consumption was found to be dominated by aeration of the activated sludge bioreactors (35-65%), mechanical pumping (26-40%), and heat supply to the digesters (5-35%). Several studies on energy analysis of WRRFs have shown the high energy utilization of these unit processes during wastewater treatment (Guerrini et al., 2017; Jonasson & Ulf Jeppsson, 2007; Merlin & Lissolo, 2010).

The simulation results suggested that AD at 35 °C with WAS ozonation, with or without thermophilic hydrolysis (35 °C/WAS HD & O_3, and 35 °C/WAS HD), led to the highest net energy consumptions among the plant configurations tested. The net energy consumption of the other configurations was between 25 and 35 % lower (Fig. 6.3). Although thermophilic hydrolysis of WAS and ozonation (35 °C/ WAS HD & O_3) led to a similar VSS destruction and biogas productions than WAS ozonation (35 °C/ WAS O_3) (Fig. 6.2), the high energy requirements and losses during heat up and cool down of the thermophilic hydrolyzer operated at 55 ºC explain most of these differences.

As discussed above, the pretreatment configurations (35 °C/ WAS HD, 35 °C/ WAS O_3, 35 °C/ WAS HD & O_3 and 20 °C/ WAS O_3) led to a higher NH_4^+-N concentration returned from the digestate than the no WAS pretreatment with digester operated at 35 ºC (35 °C/ No HD & No O_3), which in turn led to a higher energy consumption by the blowers of the aeration tanks of the main water treatment. However, this increase in blower energy consumption was compensated by the higher biogas production (35 °C/ WAS O_3 and 35 °C/ WAS HD & O_3), or the combination of lower heat requirement and higher biogas production (20 °C/ WAS O_3). This suggests that designers should not worry about the possible negative effect on the net energy balance of the plant due to a higher NH_4^+-N return because it was not observed in the simulations. Thus, the major difference between the digesters operated at 35 ºC and 20 ºC was the significant heat requirement for increasing the temperature of the digester content in the high mesophilic temperature reactors

and the additional energy required for activated sludge aeration. By substituting thermal energy with ozone to pretreat biosolids, it was possible to operate digesters at low mesophilic temperature (20 ºC) and reduce the energy footprints of AD installations.

Energy balance simulations and models provide useful insights of the energy flow in anaerobic digestion systems, but they also involve uncertainties. Kops and Vanrolleghem (1996) studied the behavior of energy balance models (EBMs) in conjunction with combined heat and power (CHP) generation systems and found that factors such as the variation in waste composition and operational parameters led to uncertainties in the model. According to Lidholm and Ossiansson (2008), uncertainties arising from modeling energy balances of anaerobic digesters combine the uncertainty of the energy model structure and parameters/measurements used to construct the model. Thus, quantification of uncertainties associated with EBMs is necessary in order to increase the accuracy of their prediction and consequently would increase the accuracy of the associated costs (EuropeanCommission, 2010).

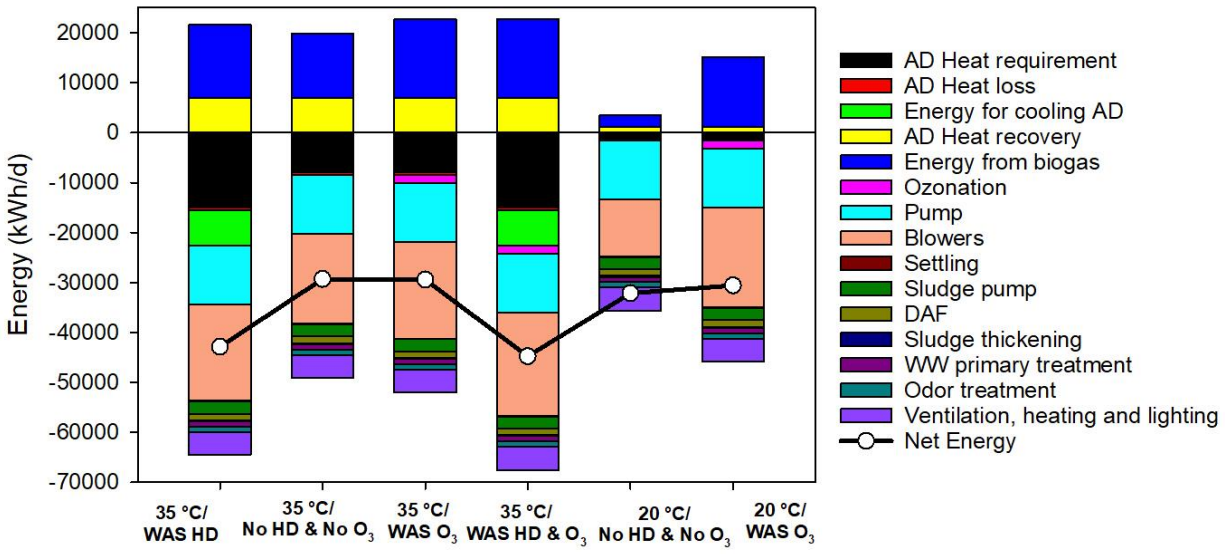

Fig 6.3. Energy budget (expenditure and gain) for different operational scenarios (20 and 35 °C, and with or without pretreatment). Stacked bars showing positive energy refer to energy gain while bars displaying negative energy relate to energy consumed by the system. The net energy of the operational configurations is shown as a solid line superimposed on the stacked bar chart.

Note: Energy for cooling AD represents the energy required for cooling down the temperature of the sludge leaving the thermophilic hydrolyzer (55 °C) and entering the AD (35 °C).

6.3.4 GHG emission and carbon footprint

Aiming to provide a well rounded evaluation of the potential benefits of WAS ozonation, we also investigated for the six configurations the plant-wide greenhouse gas (GHG) emissions by considering both direct emission comprised of CO_2, CH_4, and N_2O generated from the treatment process units (activated sludge and anaerobic digestion) and sludge storage, and indirect emissions comprised of CO_2 sources arising from electricity consumption, chemical use for sludge dewatering and ozonation, sludge transport and disposal, with details listed in Table S6.5. Generally, total direct emissions accounted for a lower fraction of the total than indirect emissions (Fig. 6.4). The largest source of direct emission was from sludge storage (71-80% of direct emissions), followed by emissions from the AD processes itself (15-21% of direct emissions). For indirect GHG emissions, our calculations suggested that sludge disposal (landfilling and land application) accounted for the largest portion (80% of indirect emissions), while electricity consumption contributed to the second largest portion (10-20% of indirect emissions). Finally, indirect GHG emissions from chemical consumption and sludge transport were negligible (only 0.9-0.13% and 0.11-0.13% of indirect emissions, respectively). Therefore, for both direct and indirect emissions, the size of the GHG fluxes was dominated by the amount of biosolids disposal, which among the various stages, the treatment of sludge and its disposal represent a major portion of the overall energy budget for a plant (Mininni et al., 2015; Tchobanoglous et al., 2013). According to Seiple et al. (2017), sludge treatment and disposal can account for 40% of the total GHG emissions from a wastewater treatment facility. Consequently, the configuration with the digester operated at 20 °C without pretreatment (20 °C / No HD & No O_3), which essentially does not reduce the amount of VSS from the WAS, resulted in the highest direct and total GHG emissions among all configurations.

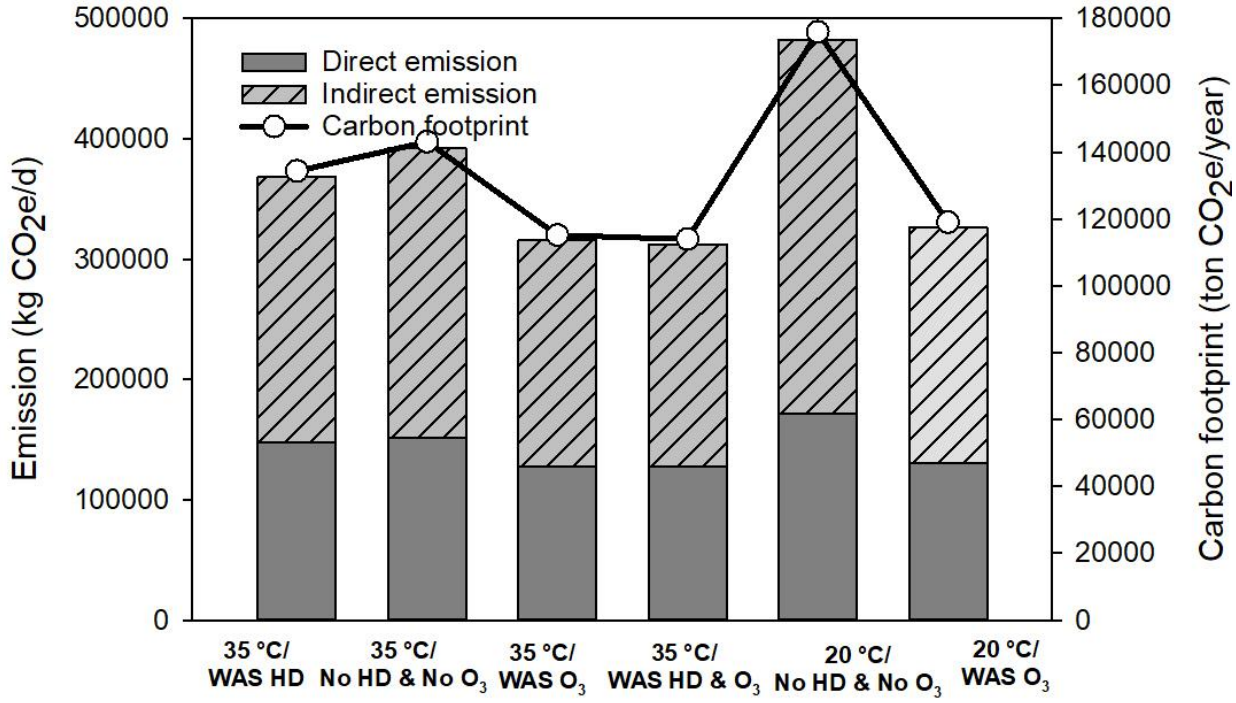

Fig 6.4. Total direct and indirect emissions for different operational scenarios of anaerobic digesters (20 °C and 35 °C, and with or without thermal hydrolysis and ozone pretreatment). The solid line depicts the total carbon footprint of the analyzed scenarios.

The net carbon footprint (direct plus indirect emissions) for the six simulated configurations were as follows: 20 °C/ No HD & No O_3 (0.868 kg CO_2e/m^3 influent) > 35 °C/ No HD & No O_3 (0.706 kg CO_2e/m^3 influent) > 35 °C/ WAS HD (0.663 kg CO_2e/m^3 influent) > 20 °C/ WAS O_3 (0.587 kg CO_2e/m^3 influent) > 35 °C/ WAS O_3 (0.568 kg CO_2e/m^3 influent) > 35 °C/ WAS HD & O_3 (0.562 kg CO_2e/m^3 influent). Thus, because of the enhanced waste biosolids reduction (i.e., VSS destruction), configurations using ozone pretreatment showed 17%-20% less GHG emissions than the conventional mode of digester operation (35 °C/ No HD & No O_3). Studies conducted on aerobic treatment systems are slightly different as compared to the results of this study, mainly due to different influent characteristics, including absence of potential carbon-based GHG emissions from constituents remaining in the treated water, emissions associated with solids disposal, or emissions from off-site electricity generation to operate the facility. Monteith et al. (2005) studied GHG emissions for 16 municipal WRRF across Canada. The emission rates ranged from 0.26 to 0.8 kg CO_2e/m^3 for conventional activated sludge with anaerobic sludge digestion.

The corresponding values obtained in this study ranged between 0.561-0.873 kg CO_2e/m^3 for a WRRF combining anaerobic digestion of pretreated biosolids (HD or O_3) at two different temperatures (35 °C or 20 °C), while considering all direct and indirect GHG emissions, including sludge disposal, electricity, transportation and chemical usage. This shows the significant impact of the type of wastewater and the indirect GHG emissions on the total GHG emissions attributed to the WRRF.

6.3.5 Economic evaluation

The operational cost of WRRFs are highly dependent on the plant configuration and influent composition (Fernández-Arévalo et al., 2017). In the present study, an economic assessment for the six operational scenarios was performed to determine the associated yearly operational cost (Fig. 6.5). The costs were subdivided into sewage and sludge treatment costs (expenditures) and income from biogas and energy recovery. For all configurations, sludge disposal, by far, had the highest operational cost followed by pumping and aeration. Therefore, similarly as with the GHG emissions, operation at 20 °C without pretreatment (20 °C/ No HD & No O_3) was found to be the most expensive option, and ozonation pretreatment of WAS to minimize biosolids production led to the lowest operational cost. The results indicate that when operating the digesters at 35 °C, the income from biogas and energy recovery was significant. The lowest positive income was obtained for 20 °C/ No HD & No O_3. From an economic perspective, 20 °C/ WAS O_3 was found to be a better option than the base case scenario (35 °C/ WAS HD). Substituting sludge hydrolysis at 55 ºC by solids ozonation at 35 ºC (35 °C/ WAS O_3) was also found to be more cost-effective than the current mode of operation at the RAEBL WRRF.

In Europe, the average costs of the different sludge disposal routes (land application, incineration and landfilling) vary from 160 Euro/tDM to more than 300 Euro/tDM (Commission, 2001; Kelessidis & Stasinakis, 2012). In the USA, the cost of land application, landfilling and incineration are estimated to be 300-800, 100-600 and 300-500 $/tDM, respectively (Peccia & Westerhoff, 2015). However, in estimating the costs associated with sludge disposal possible uncertainties exist, such as sludge disposal route, the quantities of sludge not meeting new regulatory requirements, and pollution prevention management and costs. Costs for storage and transport of sludge is strongly affected by fuel, labor costs, and distance from the treatment plant

to the final disposal destination, which vary widely depending on the local conditions and economics.

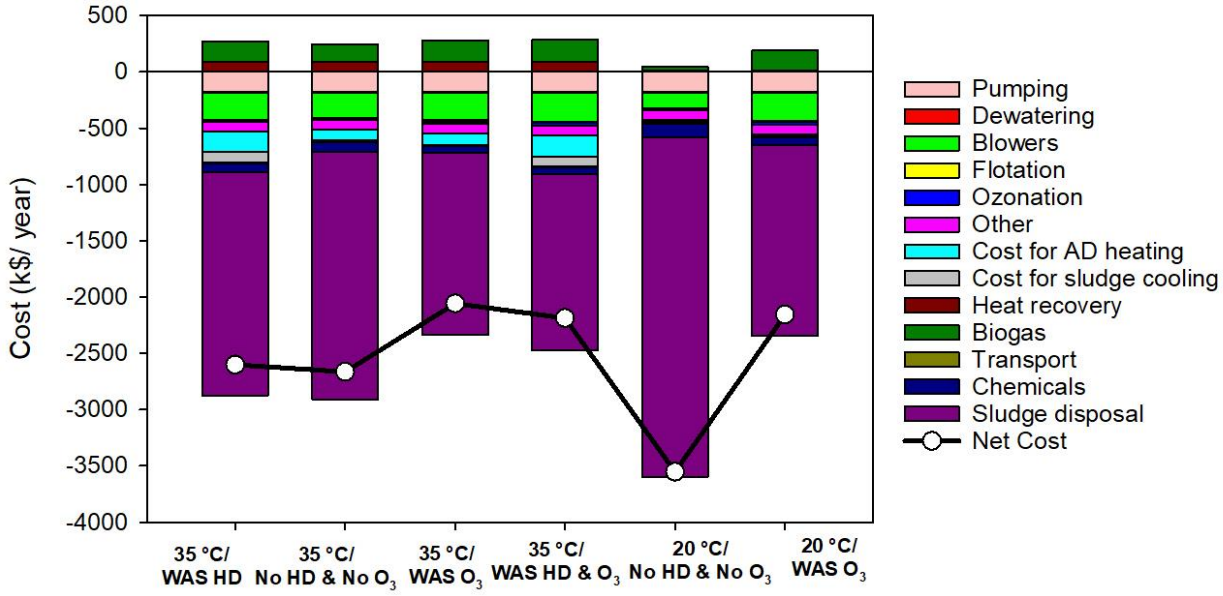

Fig 6.5. Operational cost analysis for different operational scenarios (20 °C and 35 °C, and with/without thermal hydrolysis and ozonation pretreatment). Positive values indicate income, while negative values represent expenditures.

6.3.6 General applicability of presented model

The RAEBL treatment plant is unusual since it has a very high proportion of non-degradable particulate COD in the influent (Table S6.1). Furthermore, the temperature profile is also specific for the simulation location and year. In order to generalize the simulation, the model was used to simulate a typical WRRF (typical municipal influent COD fractions, constant influent concentrations, and constant temperatures for winter and summer) with a typical full-scale digester operated at 35 ºC with raw WAS (35 °C/ No HD & No O_3) and at 20 ºC with WAS ozonation (20 ºC/WAS O_3). The typical WWRF was defined as the default values from BioWin v5.2 (Table S6.1 & Table S6.10). The performance of the digesters was assessed over a winter and summer season. The resulting outputs may provide plant operators and decision makers/investors with a quick outlook on the potential benefits of implementing a combined system of AD with sludge ozonation at mesophilic temperature (20 °C) (Table 6.2).

From a digestion performance perspective, the 20 °C/ WAS O_3 digester had almost 22% and 29% higher VSS reduction, and 33% and 25% higher biogas production for winter and summer, respectively, compared to the conventional AD at 35 ºC. These modeling results are in line with the results obtained in our previous study (Table 6.2b; Chapter 3) The enhanced conversion performance due to WAS ozonation, coupled with operation at 20 °C, led to an overall improvement in energy recovery of 34% and 24% for simulated winter and summer conditions, respectively (Table 6.2a). However, the increase in aeration requirements for the nitrification of the returned ammonium essentially used all the extra energy recovered, and the plant-wide energy balance was essentially unchanged (Table 6.2a).

Finally, enhancing anaerobic digestion of WAS reduces GHGs emissions beyond what is achieved by the 35 °C/ No HD & No O_3 basic scenario for both seasons. Consequently, the lower carbon footprint of such an installation represents a more sustainable option for treating municipal sludge.

Table. 6.2. Comparison of digester performance, energy analysis and carbon footprint of typical full scale WRRF (default influent COD fractionations, kinetic and stoichiometric values from BioWin) for anaerobic digester at 35 °C fed with raw WAS and anaerobic digester at 20 °C fed with ozonated WAS.

Criteria	Parameter	Unit	35 °C/No HD & No O_3		20 °C/ WAS O_3	
			Winter*	Summer*	Winter	Summer
a) Plant wide model performance						
Digester performance	Biogas production	m^3/ h	98.8	91.3	131.5	114.6
	Comparison with conventional operation				+33.1%	+25.5%
	VSS	mg/ L	27,011	26,501	21,957	21,875
	VSS reduction	%	37.0	34.0	45.0	44.0
	Comparison with conventional operation				+21.6%	+29.4%
	NH_4^+	mg/ L	1,522	1,326	1,848	1,621
	Comparison with conventional operation				+21.4%	+22.2%
Digester energy performance	Heat requirement	kWh/ d	–7,456	–5,070	–2,386	0
	Heat loss through walls, roof and floor	kWh/ d	–764	–301	–492	–29
	Energy for ozonation	kWh/ d	0.0	0.0	–1,326	–1,326
	Energy from biogas	kWh/ d	+15,646	+14,506	+20,831	+18,212
	Heat recovery	kWh/ d	+6,562	+4,462	+2,100	0
	Overall net energy balance for AD	kWh/ d	+13988	+13597	+18727	+16857
	Comparison with conventional operation				+34%	+24%
Other WRRF unit energy performance	Energy for activated sludge blowers	kWh/ d	–18,890	–18,573	–22,937	–22,553
	Energy for Equipment (Pump, ventilation, etc.)	kWh/ d	–29,711	–29,275	–29,711	–29,275
	Overall net energy balance for other WWRF units	kWh/ d	–48,601	–47,848	–52,648	–51,828
	Comparison with conventional operation				–8.3%	–8.3%
Energy gain	Biogas and heat recovery	kWh/ d	+22,208	+18,967	+22,930	+18,212
Energy expenditure	Heat requirement, heat loss, ozonation and blowers & equipment	kWh/ d	–56,821	–53,219	–56,852	–53,183
Total net energy balance	Overall Net energy (Energy expenditure –Energy gain)	kWh/ d	–34,613	–34,252	–33,922	–34,971
	Comparison with conventional operation				–2.0%	+2.1%

GHG emission & Carbon footprint	Total Indirect emission (Electricity, transportation, chemicals, sludge disposal)	kg CO_2e/ d	130,130	127,813	118,167	117,194
	Total Direct emission (activated sludge process, effluent discharge, sludge storage and anaerobic digestion)	kg CO_2e/d	128,747	119,803	115,612	107,884
	Carbon footprint	t CO_2e/ year	94,490	90,380	85,329	82,153
	Comparison with conventional operation				–9.7%	–9.1%
Criteria	Parameter	Unit	35 °C/No HD & No O_3 **		20 °C/ WAS O_3 **	
b) Anaerobic digester performance (Chapter 3).						
Digester performance	Biogas production	mL/week		925±15		1,045±6
	Comparison with conventional operation					+13%
	VSS	mg/ L		5,525±56		3,782±49
	VSS reduction	%		44±2		61±3
	Comparison with conventional operation					+39%
Digester energy performance	Heat requirement	kWh/ d		–41,667		–16,667
	Heat loss through walls, roof and floor	kWh/ d		–7,778		–3,056
	Energy for ozonation	kWh/ d		-		–10,278
	Energy from biogas	kWh/ d		+62,778		+75,833
	Heat recovery	kWh/ d		+33,334		+13,334
	Energy for Mixing	kWh/ d		–10,833		–10,833
Energy gain	Biogas and heat recovery	kWh/ d		+96,112		+89,167
Energy expenditure	Heat requirement, heat loss, ozonation and mixing	kWh/ d		–60,274		–40,834
Total Net energy	Overall Net energy (Energy expenditure –Energy gain)	kWh/ d		+35,838		+48,333
	Comparison with conventional operation					+35%

*Average ambient temperature for winter: -15 °C and for summer: 20 °C

** Average ambient temperature: 10 °C

6.4 DISCUSSION

6.4.1 Ranking performance of anaerobic digestion configurations

This study further investigated the feasibility and benefits of ozonation of WAS before anaerobic digestion and the reduction of the digester operation temperature to 20 °C. Results from Chapters 3 and 5 experimentally established the technical feasibility of this approach. Furthermore, AD at low mesophilic or even psychrophilic temperatures has been shown possible for substrates such as wastewaters, manure, soil and slurries (Liu et al., 2016; Martí-Herrero et al., 2015; Massé et al., 1996). However, lower temperatures require longer SRT, or an increase in digester footprint, to achieve optimal gas production (Rittmann & McCarty, 2001a). According to Li and Jha (2014), the biogas production by a digester operating at 20 ºC and retention time of 40-50 days is comparable to a digester operating at high mesophilic temperature. The approach investigated, maintain the same design SRT as for 35 ºC, which we hypothesized could contribute to enhanced performance by the digester.

Going beyond our previous work, we wanted to evaluate the impact of the new technology from a plant-wide perspective. Four criteria were used to evaluate the performance of the various WAS anaerobic digester configurations: digestion performance, plant-wide energy utilization, plant-wide carbon footprint, and plant-wide operational cost. The ranking of digester configurations is summarized in Table 6.3. The configurations 35 °C/ WAS O_3 and 20 °C/ WAS O_3 are the only two that ranked among the highest for all criteria. From the plant-wide perspective, the two temperature configurations are very similar (digestion performance (55.8% and 55.4% of VSS destruction), plant-wide energy utilization (–29,425 and –30,605 kWh/d), plant-wide carbon footprint (188,456 and 196,305 kgCO_2e/d), and plant-wide operational cost (2,056 and 2,157 k$/yr) for 35 °C/ WAS O_3 and 20 °C/ WAS O_3, respectively). The main driver explaining the better performance of these configurations over the others is the enhancement of VSS destruction and methane conversion by the WAS pretreatment. Nonetheless, the slight difference in the performance between the two operational temperatures of the digesters at full-scale level might involve some uncertainties, which consequently might produce a different result.

This analysis provides insights for decision making and evaluating operational options by considering several criteria related to plant sustainability. Thus, it should be emphasized that the objective of this study was not to predict all the criteria values with absolute accuracy, but to

provide a better comparative picture of the overall WRRF performance, incorporating anaerobic digestion at low mesophilic temperature and WAS ozonation. With the use of this analysis it is now possible to see how effluent standards, energy performance and the causes of GHG emissions are entangled. Also, the simulation values need to be interpreted with care as the GHG emission factors and operation costs were taken for the province of Quebec and they could be different elsewhere. Also, some possible GHG sources are not completely listed, such as formation of CH_4 in the sewer system (Guisasola et al., 2009) and stripping afterwards in the treatment plant (influent, pumping station, aeration tank). The evaluation of the economic impacts of the different operational scenarios was restricted to the operational costs, therefore excluding costs related to infrastructure (capital expenditures of new technology, treatment device replacements) and maintenance (personnel costs). These additional data can have a significant influence on decision-making during the construction phase as highlighted by Landry and Boyer (2016). Inclusion of capital expenditures for a large-scale technology can be very challenging and may be carried out at a later stage of the new technology development and implementation. Consequently, the reader should keep in mind that the results of this study depend on the assumptions made by the authors as presented in the methods section.

Table 6.3. Ranking performance of the six configurations for four different indicators

Indicators	Digester configuration ranking order		
	High	Medium	Low
Digester performance (VSS reduction, biogas production, energy recovery)	• 35 °C/ WAS HD & O_3 • 35 °C/ WAS O_3 • 20 °C/ WAS O_3	• 35 °C/ WAS HD • 35 °C/ No HD & No O_3	• 20 °C/ No HD & No O_3
Plant-wide energy utilization	• 35 °C/ No HD & No O_3 • 35 °C/ WAS O_3 • 20 °C/ WAS O_3 • 20 °C/ No HD & No O_3	• 35 °C/ WAS HD • 35 °C/ WAS HD & O_3	
Plant-wide GHG emission & carbon footprint	• 35 °C/ WAS HD & O_3 • 35 °C/ WAS O_3 • 20 °C/ WAS O_3	• 35 °C/ WAS HD • 35 °C/ No HD & No O_3	20 °C/ No HD & No O_3
Plant-wide operation cost	• 35 °C/ WAS HD & O_3 • 35 °C/ WAS O_3 • 20 °C/ WAS O_3	• 35 °C/ WAS HD • 35 °C/ No HD & No O_3	20 °C/ No HD & No O_3

Note: Digester performance ranking: High (higher VSS reduction and biogas production) Low (lower VSS reduction and biogas production); Energy performance: High (lower net energy) Low (higher net energy); GHG emission & Carbon footprint: High (lower emissions) Low (higher emissions); Cost: High (lower net cost) Low (higher net cost).

6.4.2 Importance of system boundary selection for energy performance analysis

The current study demonstrates how choosing the system boundary will greatly impact the conclusions about the effect of a new process unit in a plant. By delimiting the system boundary around the anaerobic digester, the 20 °C/ WAS O_3 scenario will be more energy sustainable with 24-34% higher net energy production, which was similar to the findings in our previous study (Table 6.2 b) (Chapter 3). Enhancing anaerobic digestion, however, comes at the price of a higher NH_4^+-N concentration in the digestate, which increases the aeration demands in the activated sludge basins (Bougrier et al., 2007; Manterola et al., 2007). Therefore, maintaining adequate aeration requires increasing blower energy usage. Consequently, by fixing the system boundary for evaluation of energy balance performance around the whole plant (Table 6.2 a), the total net energy usage is essentially not changed by enhancing digestion by ozonation of WAS.

6.4.3 VSS destruction and sludge disposal: drivers of GHG emissions and operational cost benefits

Sludge production and disposal result in serious environmental issues including emission of GHGs, which contribute to climate change. According to Seiple et al. (2017), sludge treatment and disposal can account for 40% of the total GHG emissions from a wastewater treatment facility. Our modeling results identified sludge treatment as the highest direct emitter of GHGs (71-80% of the total direct GHG emissions), and sludge disposal at landfill sites and agricultural lands as the main source of indirect GHG emission (almost 80% of total indirect GHG emissions). After landfilling or field application of biosolids, GHG emissions have been found to be dominated by N_2O, whereas CH_4 is of minor importance since most of the degradable organic carbon has been turned into biogas (Wulf et al., 2002). This is significant since N_2O has a global warming potential (GWP) 298 times greater than CO_2 over a hundred years (IPCC, 2006). Hence, enhancing VSS destruction with WAS ozonation improved GHG emissions.

Analysis of the operational costs revealed that sludge disposal and mechanical pumping were the highest budget items for all operational scenarios. Specifically for the province of Quebec, the imposition of the landfill disposal tax has triggered a dramatic increase in the disposal costs of sewage biosolids from $30/ton in 2006 to over $100/ton in 2018 (LeBlanc et al., 2009; RECYC-QUÉBEC, 2018). Hence, strategies to reduce waste biosolids production effectively curbs down operational costs. Thus, operational cost reduction was also highest with WAS ozonation that

maximized the VSS reduction. Although the aeration cost increased under the configurations involving sludge ozonation, it was offset by a much higher reduction in sludge disposal cost.

Specifically for the RAEBL, based on the operational cost evaluation, operating the anaerobic digesters at 20 ºC with sludge ozonation (20 °C/WAS O_3) entailed a much lower cost (by 443,935 $/yearly) than the current mode of operation involving AD at 35 ºC with only thermal hydrolysis (35 °C/ WAS HD). Hence, operating a hybrid AD-sludge ozonation system may prove more advantageous from an economic perspective. The current modeling approach has identified options for optimizing processes of the RAEBL WRRF in view of improving its cost benefit. Operating the digestion system, currently in place, at 20 ºC with sludge pretreatment by ozone instead of 35 ºC with the hydrolyzer unit, can enable the facility to save up to 30% of energy and reduce the cost. This energy conservation is significant and can be an extremely effective way of reducing and optimizing cost at the RAEBL WRRF.

6.5 CONCLUSION

The plant-wide models presented in this study makes up a useful engineering tool to aid decision makers to improve the sustainability and efficiency of wastewater treatment systems involving AD of municipal sludge. Based on the simulation results, integrating sludge ozonation was found to impart added benefits to AD including feasibility at low mesophilic temperature (20 ºC), enhanced digester performance in terms of VSS reduction and biogas production, higher energy sustainability, and reduced carbon footprint and operational cost, than in conventional AD at 35 ºC. The assessment of a representative full-scale treatment facility, in this case the RAEBL WRRF, has provided evidence that the proposed new configuration combining sludge ozonation at low mesophilic temperature presents higher energy efficiency as well as environmental benefits in terms of lower direct and indirect GHG emissions and reduced economic impacts. Modification of the existing sludge treatment line of the RAEBL WRRF to integrate sludge ozonation and low temperature AD can be foreseen to validate the developed plant-wide model and reduce uncertainties, especially considering that the facility already houses an ozone unit for disinfecting its treated effluent. Implementing redundancy in its operation to ozonate secondary sludge prior to AD at low mesophilic temperature can help optimize its treatment capacity, energy efficiency, carbon footprint, and operational costs. These advantages, and the potential of achieving more environmental and economic benefits, represent promising arguments for low temperature AD of

ozonated municipal sludge, even if they require redesign of the treatment line. The future development of this proposed technology would allow for refining the sustainability assessment and, therefore, better substantiating the arguments for its commercialization. Meanwhile, the application of plant-wide modeling and simulation tools to systematically analyse different operational scenarios through extrapolation of lab-, pilot- and full-scale data represents a powerful means of identifying treatment strategies with enhanced environmental and economic impacts.

6.6 ACKNOWLEDGEMENTS

The authors thank the operators of the wastewater treatment facilities for granting permission to collect samples. The research was supported by grants from the Natural Sciences and Engineering Research Council (NSERC) of Canada (NSERC CRDPJ 5008), Air Liquid Canada and the Régie d'Assainissement des Eaux du Bassin LaPrairie (RAEBL). The project was also supported by the Mathematics of Information Technology and Complex Systems (MITACS) of Canada.

6.7 SUPPORTING INFORMATION

Table S6.1. Influent fractions and model parameters used for simulation study.

Parameters	Units	Calibrated value	Default value
Influent COD organic fractions			
Soluble biodegradable organics (f_{SB})	g-COD_{SB} / g-Total COD	0.200[a]	0.160
Particulate undegradable organics (f_{XU})	g-COD_{XU} / g-Total COD	0.550[a]	0.130
Soluble undegradable organics (f_{SU})	g-COD_{SU} / g-Total COD	0.080[a]	0.050
Slowly biodegradable substrates (f_{XCB})	g-COD_{XCB} / g-Total COD	0.170[a]	0.660
Ordinary heterotrophic biomass (f_{XOHO})	g-COD_{XOHO}.m^{-3}	0.020[b]	0.020
Nitrifying biomass (f_{XANO})	g-COD_{XANO} / g-Total COD	0.01[d]	0.0001
Influent fractions			
Particulate undegradable (X_U)/VSS ratio	g-COD/g-VSS	1.630[a]	1.600
VSS/TSS ratio	g-VSS/g-TSS	0.800[a]	0.920
Composition coefficients			
N- content of endogenous residue	g-N/g-COD	0.068[d]	0.070
P-content of endogenous residue	g-P/g-COD	0.021[d]	0.022
Biological parameters			
Yield of ordinary heterotrophic biomass (Y_{OHO})	g-COD/g-COD	0.670[c]	0.660
Acetoclastic yield	g-COD/g-COD	0.1[b]	0.100
Methanol acetoclastic yield	g-COD/g-COD	0.1[b]	0.100
H_2-utilizing yield	g-COD/g-COD	0.1[b]	0.100
Methanol H_2-utilizing yield	g-COD/g-COD	0.1[b]	0.100
Heterotrophic max. specific growth rate ($\mu_{OHO,Max}$)	d^{-1}	3.200[b]	3.200
Acetoclastic max. spec. growth rate	d^{-1}	0.300[b]	0.300
H_2-utilizing max. spec. growth rate	d^{-1}	1.400[b]	1.400
Acetoclastic substrate half sat	mgCOD/L	100.0[b]	100.0
Acetoclastic methanol half sat	mgCOD/L	0.500[b]	0.500
H_2-utilizing CO_2 half sat.	mmol/L	0.100[b]	0.100
H_2-utilizing substrate half sat.	mgCOD/L	1.000[b]	1.000
H_2-utilizing methanol half sat	mgCOD/L	0.500[b]	0.500
Anaerobic hydrolysis factor	-	2.000[b]	2.000
Heterotrophic decay rate	d^{-1}	0.620[b]	0.620
Endogenous decay rate	d^{-1}	2.000[b]	2.000
Ammonification rate (q_{am})	m^3/g-COD/d	0.080[b]	0.080
Hydrolysis rate	g-X_{CB}/g-X_{OHO}/d	2.000[b]	2.000
Transformation parameters by ozone			
Fraction of soluble undegradable COD from biomass ($f_{SU\_O3,trans}$)	g-COD_{SU}.g-COD_X^{-1}	0.216[e]	

Fraction of soluble biodegradable COD from biomass ($f_{SB\_O3,trans}$)	$g\text{-}COD_{SB}.g\text{-}COD_X^{-1}$	0.418 [e]
Fraction of particulate biodegradable COD from biomass ($f_{XCB,O3,trans}$)	$g\text{-}COD_{XCB}.g\text{-}COD_X^{-1}$	0.366 [e]
Fraction of oxidized COD ($f_{mnr,O3}$)	$g\text{-}COD_{mnr.g}\text{-}COD_X^{-1}$	0.040 [e]

[a]: Calibrated from historical data.
[b]: Default value from Biowin 5.2 (Envirosim, 2017).
[c]: Model value adopted from (Hauduc et al., 2011).
[d]: Values obtained from (Jauffur, 2016).
[e]: Values adapted from (Isazadeh et al., 2014).

Table S6.2. Energy consumption of different equipment's in RAEBL WRRF

Equipment	Average seasonal energy consumption (kWh/d)			
Aerobic system	Winter	Spring	Summer	Fall
Lift pumps	3148	3020	3808	3734
Lift pumps	4896	4697	5923	5808
Primary treatment	986	946	1193	1170
Blowers	17691	16971	21401	20987
Recirculation pump	1237	1186	1496	1467
Secondary sludge extraction	108	104	131	128
Secondary settling	27	26	33	32
Effluent pump	1607	1542	1944	1906
Pressurization pump	775	744	938	920
Pump of thicken sludge	786	754	951	93
Pump of thicken sludge	8	78	98	9
Pump of thicken sludge	90	86	109	107
Pump of excess sludge	72	69	87	86
Pump of excess sludge	18	17	22	21
Pump of sludge in holding tank	9	9	11	11
Pump of polymer for dehydration	414	39	50	492
Dehydration	48	46	58	57
Air compressor (thickener and dehydrator)	70	67	85	83
Air compressor (rectangular thickener)	302	289	365	358
Air booster (holding tank)	108	10	131	129
Odor treatment	775	744	938	920
Ventilation, heating and lighting	1020	978	1234	1210
Anaerobic system				
Transfer pump to HD	25	25	25	25
Mixing pump of HD	56	56	56	56
Pump A circulator hydrolyser	200	187	16	187
Mixing pump of AD1	148	148	148	148
Pump B circulator AD1	91	108	110	108
Mixing pump of AD2	148	148	148	148
Pump C circulator AD2	91	108	110	108
Biogas blower	45	45	45	45
Pump D for circulation of hot water from the exchanger to the AD	286	286	286	286

Table S6.3. Components of energy flow model in anaerobic digesters

Energy component	Mathematical equation	Eq. Number
Heat requirement	$E_{req} = Q\left(T_{dig/hydro} - T_{In}\right)\rho_{sludge} \cdot c_{sludge}$	S1
Heat loss through walls, roof and floor	$E_{loss} = E_{loss,wall} + E_{loss,floor} + E_{loss,cover}$ $E_{loss,i} = A_i\left(T_{dig/hydro} - T_{Air\ or\ ground}\right) h_i$	S2
Energy for cooling digester	$E_{cooling} = Q\left(T_{hydro} - T_{dig}\right)\rho_{sludge} \cdot c_{sludge}$	S3
Heat recovery	$E_{rec} = Q\left(T_{exc-hot} - T_{exc-cold}\right)\rho_{sludge} \cdot c_{sludge} \cdot \eta$	S4
Energy for ozonation	$E_{O3} = O_3\ dose \times m_{solids} \times Elec_{O_3}$	S5
Energy from biogas	$E_{CH_4} = V_{CH_4} \times 9.95\ \text{kWh}/m^3$	S6

$Elec_{O3}$=12.5 kWh/ kg O_3; h_i = wall=0.23, floor=0.57 and cover=0.23 W/m^2 °C; Q = 327 m^3/d; T_{dig} = 37 or 20 °C; T_{in} = -12.5 °C, 17 °C, 20 °C and 17 °C for winter, spring, summer and fall, respectively; $T_{exc\text{-}hot}$ = 55 °C; $T_{exc\text{-}cold}$ = 37 or 20 °C; T_{Air} = -15 °C, 12 °C, 25 °C and 12 °C for winter, spring, summer and fall, respectively; T_{ground} = -3 °C, 5 °C, 15 °C and 5 °C for winter, spring, summer and fall, respectively; ρ_{sludge} = 1,026 kg/m^3; c_{sludge} = 4,186 J/kg °C; η = 0.88

Table S6.4. Amount of sludge transported to disposal sites and their respective distance (in km) from the RAEBL plant

Period (2017)	Amount of sludge disposed (tons)					
	Landfill Sites		Landscaping Material	Land Application		
	Saint-Nicéphore	Sainte-Sophie	Black Lake	Godmanchester	Mirabel	Ryan Noyan
July	194	349	581	127		
August	305	771	253			
September	118	561	99	122		
October	84	805	17	215		
November	465	424	232			
December	205	325	306		23	25
Distance (km)	128	67	238	70	55	50

Table S6.5. Sources of direct and indirect GHG emissions for different WRRF scenarios integrating ozone and thermal hydrolysis pretreatment of biosolids combined with anaerobic digestion

Emission (kg CO_2e/d)		Scenario					
		1	2	3	4	5	6
		35 °C/WAS HD	35 °C/ No HD & O_3	35 °C/ WAS O_3	35 °C/ WAS HD & O_3	20 °C/ No HD & O_3	20 °C/ WAS O_3
Direct Emission	Gas phase (N_2O in CO_2e)	9,824 (6.7 %)	7,340 (4.8%)	8,729 (6.9%)	10,459 (8.2%)	6,072 (3.5%)	8,585 (6.6%)
	Gas phase (CH_4 in CO_2e)	26,466 (18.0%)	22,767 (15.0%)	24,902 (19.6%)	26,319 (20.6%)	5,023 (2.9%)	23,991 (18.5%)
	Liquid phase effluent (CH_4 in CO_2e)	12 (0.0%)	33 (0.0%)	20 (0.0%)	12 (0.0%)	24 (0.0%)	30 (0.0%)
	Liquid phase effluent (N_2O in CO_2e)	441 (0.3%)	285 (0.2%0	303 (0.2%)	445 (0.3%)	239 (0.1%)	304 (0.2%)
	Storage (CH_4 in CO_2e)	110,504 (75.0%)	121,312 (79.9%)	93,147 (73.3%)	90,447 (70.8%)	160,577 (93.4%)	96,969 (74.7%)
	TOTAL	147,247	151,737	127,101	127,682	171,935	129,879
Indirect Emission	Electricity (CO_2e)	36,794 (16.7%)	35,485 (14.8%)	37,840 (20.1%)	39,124 (21.2%)	29,855 (9.6%)	38,374 (19.5%)
	Transport (CO_2e)	290 (0.1%)	255 (0.1%)	209 (0.1%)	229 (0.1%)	273 (0.1%)	179 (0.1%)
	Chemicals (CO_2e)	257 (0.1%)	286 (0.1%)	210 (0.1%)	203 (0.1%)	392 (0.1%)	221 (0.1%)
	Sludge disposal:	183,506 (83.1%)	204,247 (85.0%)	150,197 (79.7%)	145,015 (78.6%)	279,598 (90.2%)	157,531 (80.2%)
	Landfill-(CH_4 in CO_2e)	13,818 (6.3%)	15,380 (6.4%)	11,310 (6.0%)	10,920 (5.9%)	21,054 (6.8%)	11,862 (6.0%)
	Land application (N_2O in CO_2e)	169,688 (76.8%)	188,867 (78.6%)	138,887 (73.7%)	134,095 (72.7%)	258,545 (83.4%)	145,669 (74.2%)
	TOTAL	220,847	240,273	188,456	184,571	310,118	196,305

OVERALL TOTAL	368,094	392,010	315,557	312,253	482,053	326,184

Emission factor: Storage= 0.12 kg CH_4/kg BOD_5 (CCME, 2009); Electricity= 0.856 kg CO_2e/ kWh (NRC, 2018b); Transport= 63.8 g CO_2e/ton-km (CNRC, 2016); Chemicals= 0.41 kgCO_2e/kgO_2 (NRC, 2017) and 2.62 kgCO_2e/ kg of sludge (Parravicini et al., 2016) for ozonation and polymer-based sludge dewatering, respectively.; Landfill= 0.13 kg CH_4/ tons of dry sludge; Land application= 0.2875 kg N_2O/ tons of dry sludge (Doka, 2003); CH_4 GWP_{100} =23; N_2O GWP_{100} = 298.

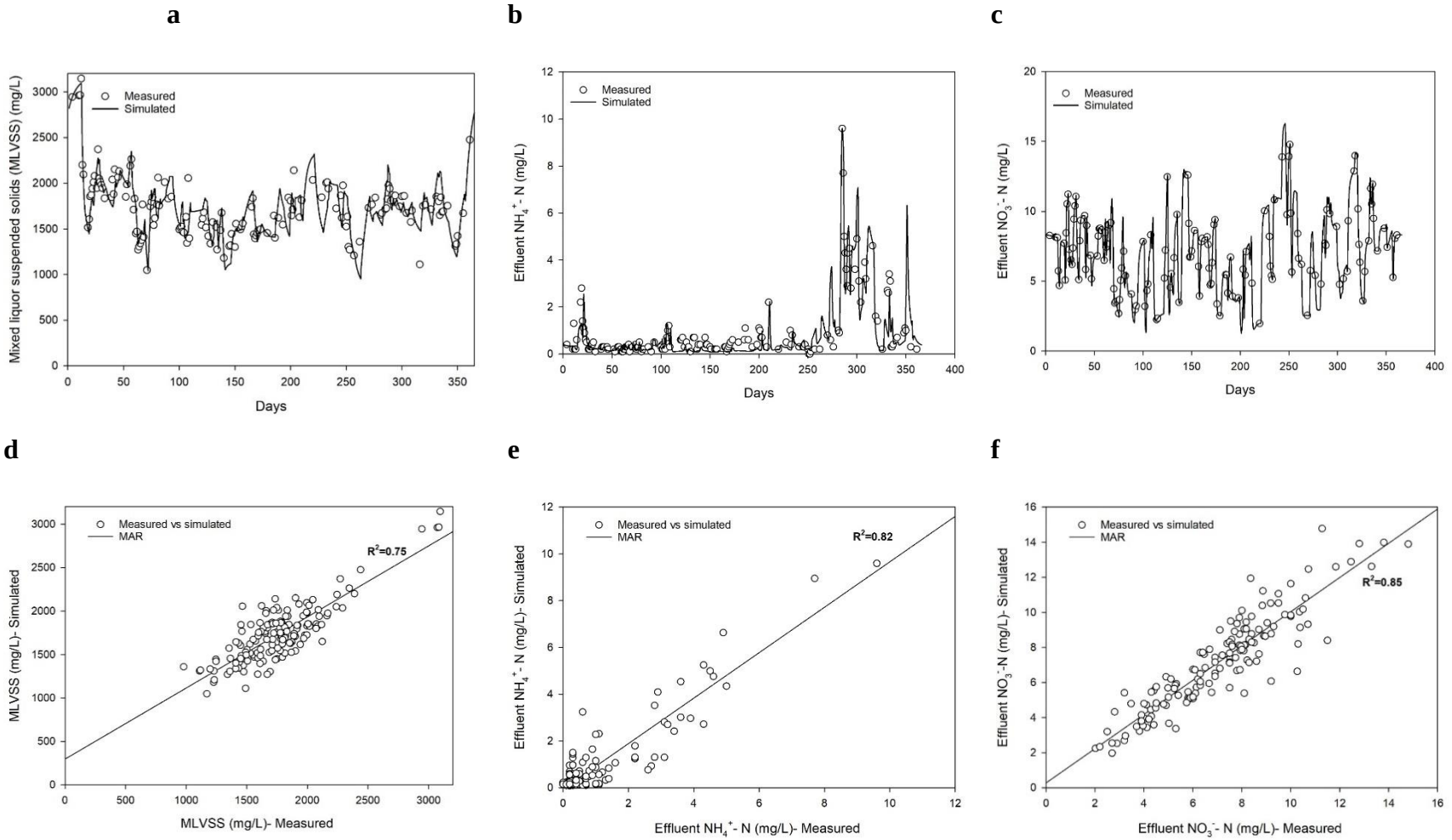

Fig. S6.1. Simulation of a) MLVSS b) effluent NH_4^+-N and c) effluent NO_3^- concentration of RAEBL WRRF. Major axis regression (MAR) of measured and predicted values for d) MLVSS e) effluent NH_4^+-N and f) effluent NO_3^- concentration. Simulation was performed using one year of operational data from Jan 1st, 2016 (Day 1) to Dec 31st, 2016 (Day 365) before the installation of anaerobic digesters.

Table S6.6. Major Axis Regression (MAR) of measured and simulated data of Fig S6.2 for one-year of operation of the RAEBL WRRF (before installation of anaerobic digesters).

Parameter	Average Parameter Value	MAR - Prediction (y-axis) vs Measured (x-axis)		
		Slope (± 95% ½ C.I.*)	Intercept [% of average value] (± 95% ½ C.I.*)	R^2
MLVSS (mg/L)	1,728	1.04±0.04	−43.47±7.72 [−2.5%]	0.75
Effluent NH_4^+-N (mg-N/L)	0.95	1.05 ± 0.03	−0.14± 0.06 [−14.7%]	0.82
Effluent NO_3^--N (mg-N/L)	7.07	1.07± 0.04	−0.41± 0.28 [−5.8%]	0.85

*MAR: Major Axis Regression; MLVSS: Mixed Liquor Volatile Suspended Solids; C.I: Confidence Interval (α=0.05); R^2: Coefficient of determination

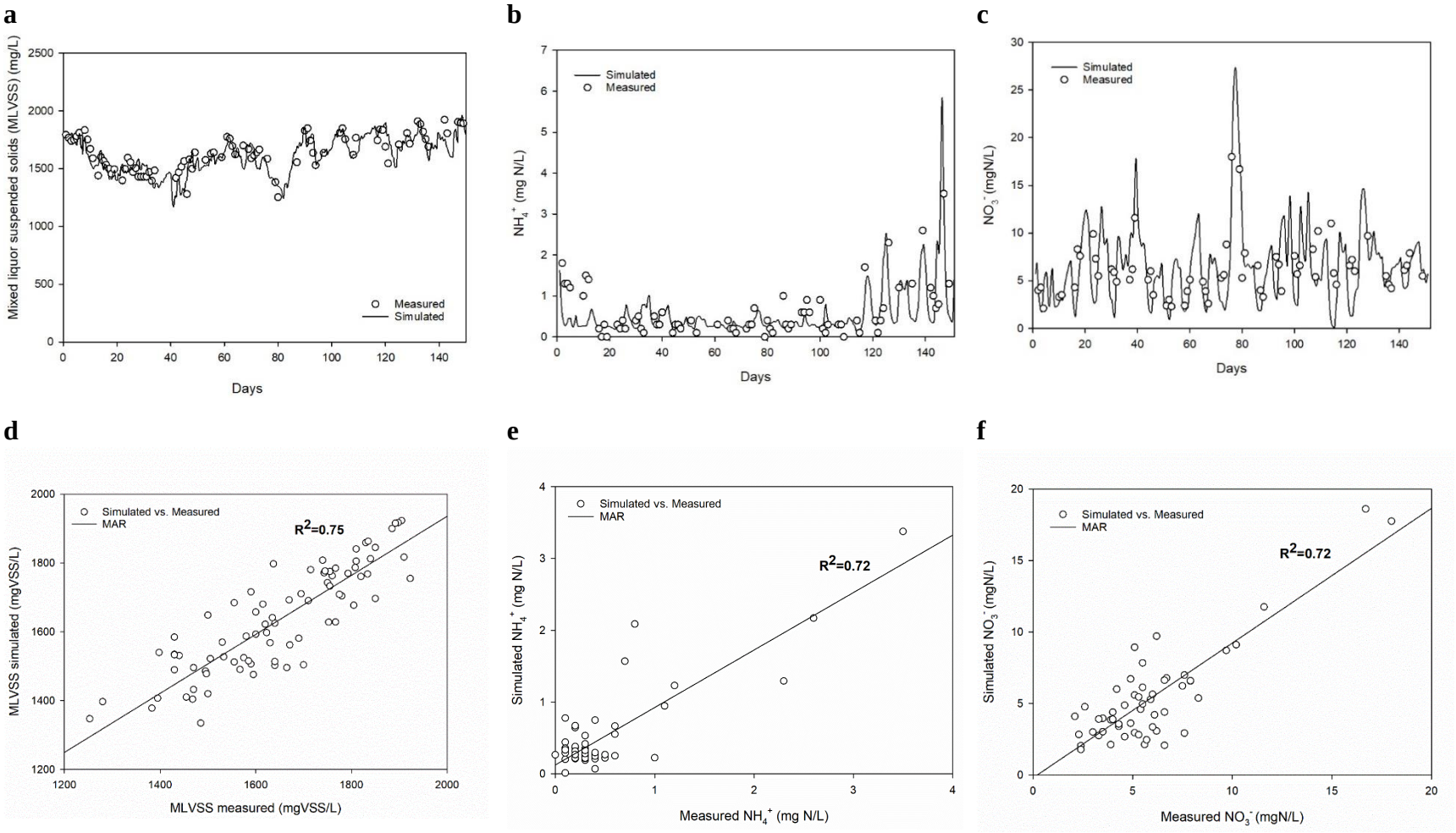

Fig. S6.2. Simulation of a) MLVSS b) effluent NH_4^+-N and c) effluent NO_3^--N concentration of RAEBL WRRF. Major axis regression (MAR) of measured and predicted values for d) MLVSS e) residual NH_4^+-N and f) residual NO_3^-. Simulation was performed using 6 months of operational data from July 22nd 2017 (Day 1) to Dec 31st 2017 (Day 150) after installation of anaerobic digesters.

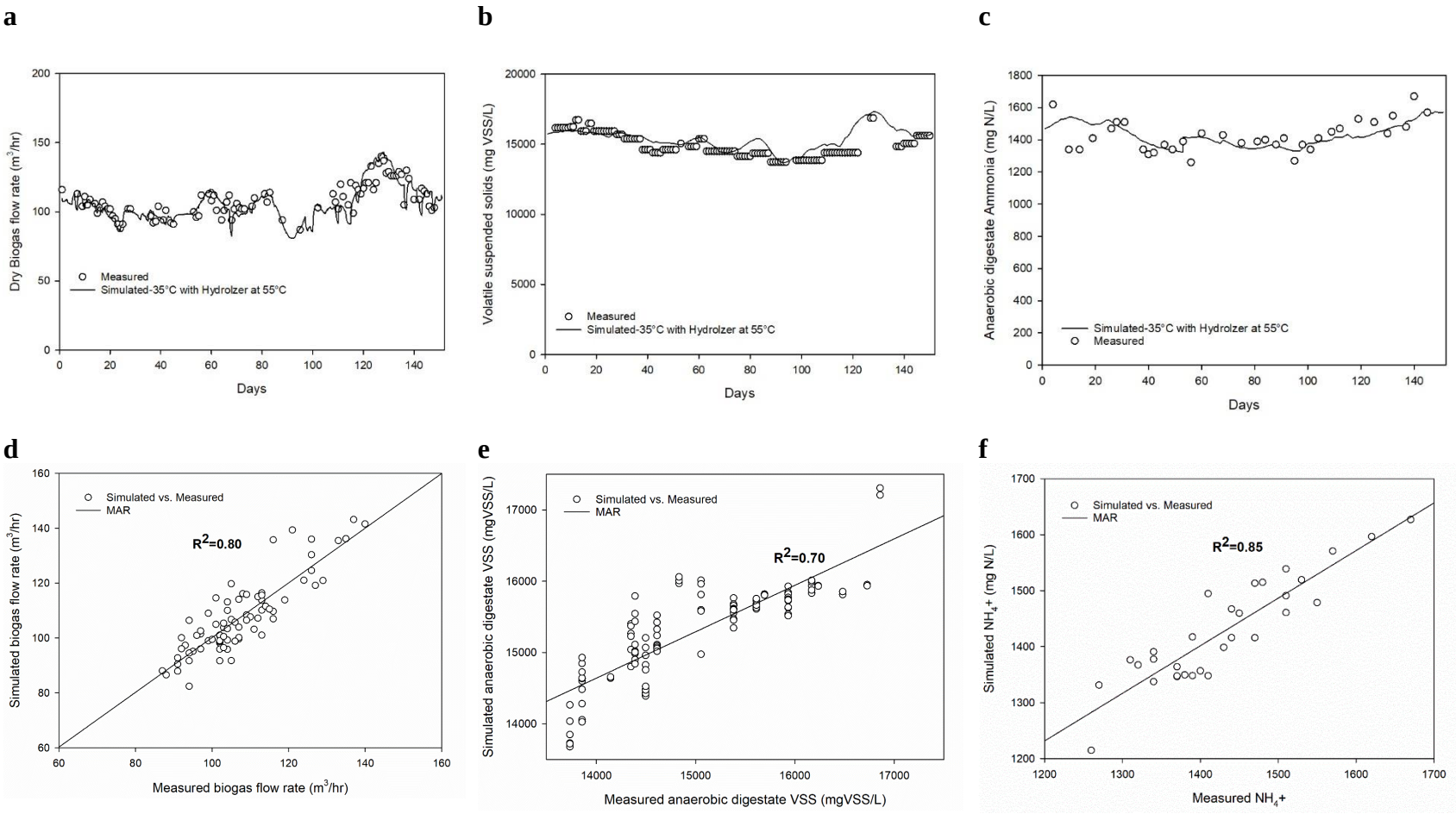

Fig. S6.3. Simulation of a) anaerobic digestate VSS b) biogas flowrate and c) digestate NH_4^+-N concentration of RAEBL WRRF. Major axis regression (MAR) of measured and predicted values for d) anaerobic digestate VSS e) biogas flowrate and f) residual digestate NH_4^+-N. Simulation was performed using 6 months of operational data from July 22nd 2017 (Day 1) to Dec 31st 2017 (Day 150) after installation of anaerobic digesters.

Table S6.7. Major Axis Regression (MAR) of measured and simulated data for Figs S6.3 and S6.4 over a period of 150 days of the RAEBL WRRF (after installation of anaerobic digesters).

Parameter	Average Parameter Value	MAR - Prediction (y-axis) vs Measured (x-axis)		
		Slope (± 95% ½ C.I.*)	Intercept (± 95% ½ C.I.*)	R^2
MLVSS (mg/L)	1,641	1.04 ± 0.06	−88.5 ± 10.05 [−5.4%]	0.75
Effluent NH_4^+-N (mg-N/L)	0.61	0.94 ± 0.07	0.05 ± 0.05 [8.1%]	0.72
Effluent NO_3^--N (mg-N/L)	6.12	1.11 ± 0.08	−0.68 ± 0.54 [−11.1%]	0.72
Digestate VSS (mg/L)	14,904	0.78 ± 0.04	2,080 ±613 [13.9%]	0.70
Digestate NH_4^+-N (mg-N/L)	1,424	0.94 ± 0.07	80.1 ± 10.7 [5.6%]	0.85
Biogas flowrate (m^3/hr)	109.0	1.14 ± 0.06	−1.55 ± 0.71 [−1.4%]	0.80

*MAR: Major Axis Regression; MLVSS: Mixed Liquor Volatile Suspended Solids; C.I: Confidence Interval (α=0.05); R^2: Coefficient of determination

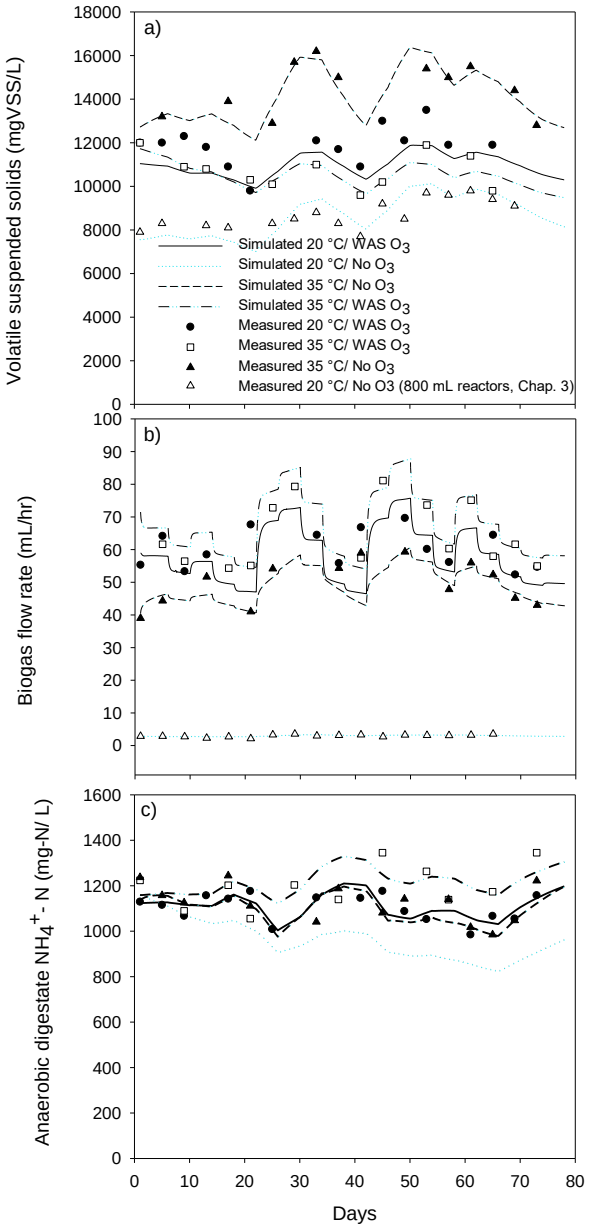

Fig. S6.4. Simulation of a) Anaerobic digestate VSS b) Biogas flowrate c) Digestate NH_4^+-N concentration for ASBRs (2000 mL, Chapter 5) operated at 20 °C and 35 °C. The solid lines and makers show the model simulations and measured data, respectively. For the scenario of AD at 20 °C with raw WAS (20 °C/ No O_3), the simulated profile was conducted on 800 mL lab scale reactors described in Chapter 3. The concentration of WAS fed to the 2,000 mL and 800 mL lab scale digesters were 30,000 and 10,000 mg/L, respectively. No measured data was available for NH_4^+ concentration of 20 °C/ No O_3 (25^{th} April-11^{th} July 2017).

Table S6.8. Major Axis Regression (MAR) of measured and simulated data over a period of 80 days (25th April-11th July 2017) of the lab-scale anaerobic digesters (Fig S6.5).

Digester	Parameter	Average Parameter Value	MAR - Prediction (y-axis) vs Measured (x-axis)		
			Slope (± 95% ½ C.I.*)	Intercept (± 95% ½ C.I.*)	R^2
20 ºC/WAS O_3	Digestate VSS (mg/L)	11,850	1.141 ± 0.190	− 1,688 ±46.6 [−14.2%]	0.70
	Digestate NH_4^+-N (mg-N/L)	1,104	0.890 ± 0.160	112.8 ± 14.4 [10.2%]	0.70
	Biogas flowrate (m^3/hr)	60.2	1.298 ± 0.187	−9.19 ± 11.3 [−15.3]	0.80
35 ºC/WAS O_3	Digestate VSS (mg/L)	10,727	1.183 ± 0.204	−1,656 ±332.0 [−15.4%]	0.75
	Digestate NH_4^+-N (mg-N/L)	1,198	0.956 ± 0.113	59.11 ± 14.07 [4.9%]	0.80
	Biogas flowrate (m^3/hr)	64.4	1.029 ± 0.104	0.586 ± 0.674 [0.91%]	0.88
35ºC/No O_3	Digestate VSS (mg/L)	14,545	1.068 ± 0.174	−1,133 ±258 [−7.8%]	0.82
	Digestate NH_4^+-N (mg-N/L)	1,124	0.996 ± 0.109	5.995 ± 0.122 [0.53%]	0.90
	Biogas flowrate (m^3/hr)	49.8	0.816 ± 0.107	8.13 ± 2.98 [16.3%]	0.85
20ºC/No O_3	Digestate VSS (mg/L)	8,713	1.362 ± 0.214	−1,033 ±157 [−11.8%]	0.75
	Biogas flowrate (m^3/hr)	2.33	1.063± 0.118	0.08±0.03 [3.4%]	0.84

*MAR: Major Axis Regression; VSS: Volatile Suspended Solids; C.I: Confidence Interval (α=0.05); R^2: Coefficient of determination

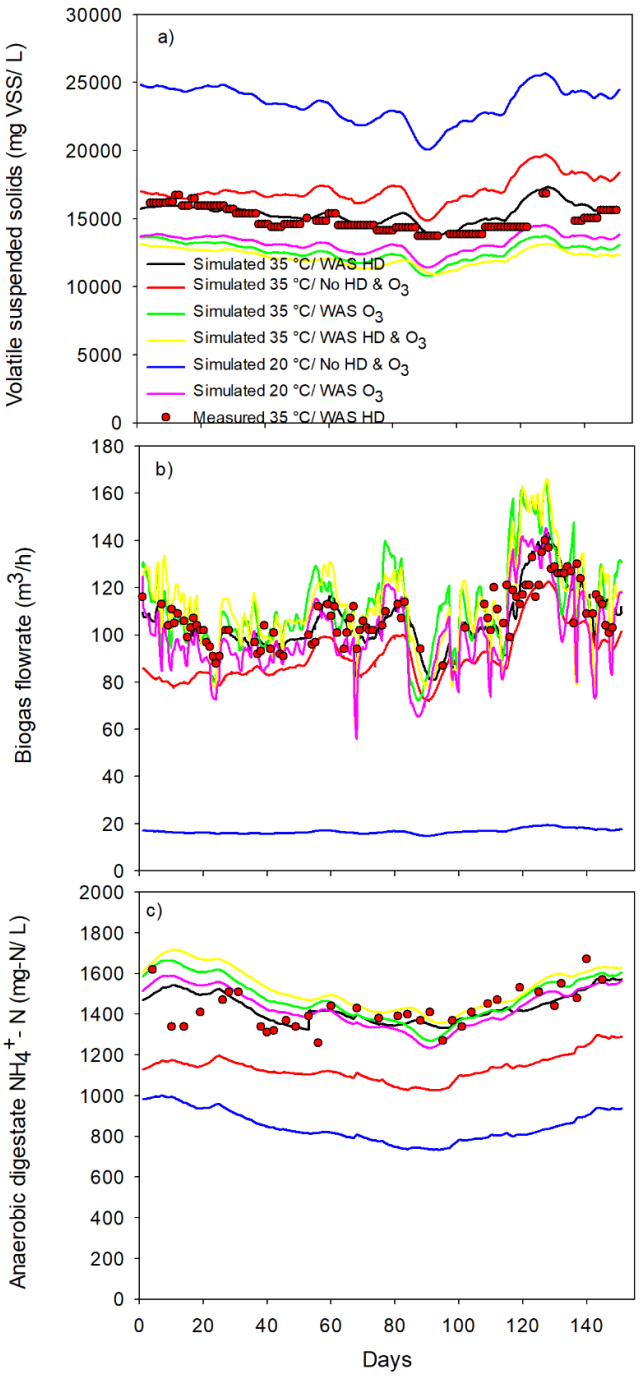

Fig. S6.5. Scenario analysis of anaerobic digesters at different temperatures (20 °C and 35 °C) and different pretreatment (thermal hydrolysis and ozonation) The solid lines show the model simulations and the red marker shows the measured data for the base case scenario (35 °C/ WAS HD).

Table. S6.9. Energy components of the treatment units for the RAEBL WRRF under six operational scenarios.

Energy component (kWh/d)	Scenario					
	1	2	3	4	5	6
	35 °C/ WAS HD	35 °C/ No HD & O_3	35 °C/ WAS O_3	35 °C/ WAS HD & O_3	20 °C/ No HD & O_3	20 °C/ WAS O_3
Heat requirement	–14,971	–7,949	–7,949	–14,971	–1,317	–1,317
Heat loss through walls, roof and floor	–596	–474	–474	–596	–202	–202
Energy for cooling digester	–7,022	0	0	–7,022	0	0
Ozonation	0	0	–1,657	-1,657	0	–1,657
Pump	–11,854	–11,854	–11,854	–11,854	–11,854	–11,854
Blowers	–19,262	–17,973	–19,307	–20,566	–11,397	–19,931
Settling	–147	–147	–147	–147	–147	–147
Sludge pump	–2,499	–2,499	–2,499	–2,499	–2,499	–2,499
DAF	–1,291	–1,291	–1,291	–1,291	–1,291	–1,291
Sludge thickening	–76	–76	–76	–76	–76	–76
Primary treatment	–1,074	–1,074	–1,074	–1,074	–1,074	–1,074
Odor treatment	–1,110	–1,110	–1,110	–1,110	–1,110	–1,110
Ventilation, heating and lighting	–4,569	–4,569	–4,569	–4,569	–4,569	–4,569
Heat recovery	6,995	6,995	6,995	6,995	1,159	1,159
Energy from biogas	14,687	12,767	15,663	15,749	2,306	14,039
Net energy	–42,867	–29,331	–29,425	–44,765	–32,148	–30,605

Note: Negative signs show energy expenditure and positive signs relates to energy gain.

Table S6.10. Operating parameters used for simulation study of the generalized model.

Parameter	Unit	35 °C/No HD & O_3		20 °C/ WAS O_3	
		Winter	Summer	Winter	Summer
Average Operating influent data					
Flow	m^3/d	60,000	60,000	60,000	60,000
Total COD	mg COD/ L	600.0	600.0	600.0	600.0
Total TKN	mg-N/ L	50.0	50.0	50.0	50.0
Total P	mg-P/ L	8.0	8.0	8.0	8.0
pH	-	0.0	0.0	0.0	0.0
Alkalinity	mmol/ L	7.3	7.3	7.3	7.3
ISS influent	mg ISS/ L	6.0	6.0	6.0	6.0
Calcium	mg-Ca/ L	45.0	45.0	45.0	45.0
Magnesium	mg-Mg/ L	80.0	80.0	80.0	80.0
Dissolved Oxygen	mg O2/ L	0.0	0.0	0.0	0.0
Aeration basin operating data					
Volume	m^3	33,600	33,600	33,600	33,600
Temperature	°C	12.0	20.0	12.0	20.0
Dissolved Oxygen	mg O_2/ L	2.0	2.0	2.0	2.0
Digester operating data					
Volume	m^3	6,200	6,200	6,200	6,200
SRT	days	20.0	20.0	20.0	20.0
Temperature	°C	35.0	35.0	20.0	20.0
Ozonation	-	No	No	Yes	Yes

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CHAPTER 7

General discussion and conclusions

Connecting text: The overall goal of this thesis was to explore the operational feasibility of anaerobic digestion (AD) of municipal biosolids at low mesophilic temperature (20 °C) combining ozone treatment. The design and implementation of such treatment process triggered a series of questions including its potential impacts on the operation of wastewater treatment facilities and on current AD modeling practices in terms of performance, energy efficiency carbon footprint and cost, which were answered during this study. In this chapter the implication of anaerobic digestion for North American markets and cold climates were investigated.

7.1 MAIN OBSERVATIONS PRESENTED IN THIS THESIS

The implementation of low mesophilic temperature (20 °C) AD combining solids ozonation was successful and resulted in a better performance in terms of volatile suspended solids (VSS) reduction and biogas production than conventional AD at 35 °C. Operation of three lab-scale anaerobic sequencing batch reactors showed that performing AD of pre-ozonated solids at 20 ºC led to a 35% higher VSS destruction and a 14% increase in methane production than AD of raw sludge at 35 ºC. Based on energy balance models, it was found that the 20 ºC-ozonated digester resulted in 35% more net energy gain than the 35 ºC digester and had a higher Energy Sustainability Index (ESI = 2.88) than the 35 ºC digester (ESI = 2.33). Assessment of AD performance by biomethane potential (BMP) assays showed a higher methane yield when using ozonated substrates at both temperatures (20 °C and 35 °C) than non-ozonated feedstocks. The application of the modified Gompertz model to study biogas production successfully predicted the ultimate methane yield resulting from the BMP assays.

High-throughput rRNA gene amplicon sequencing analyses revealed distinct bacterial and archaeal community structures and composition between digesters fed with ozonated and non-ozonated substrates at both temperature regimes. Digesters operated at low temperature (20 °C) and fed with ozonated substrates displayed a high dominance of *Clostridium*, while digesters at 35 °C with untreated feedstock showed a higher abundance of *Sedimentibacter*. The feed type and temperature, hence, represented important factors determining microbial diversity and community structure, which are closely linked to functional stability and performance of AD systems. Ozonating the anaerobic digestate rather than the waste activated sludge (WAS) was found to be a better operational strategy since it led to approximately 10% increase in VSS reduction and biogas production. The point of ozonation is, thus, an important parameter to consider when implementing low temperature AD combining ozonation. Variation in solids retention time (SRT) clearly affected the reactor performance due to accumulation of volatile fatty acids (VFAs) at low SRTs. Decoupling the SRT from the hydraulic retention time (HRT) significantly improved the VSS reduction and methane yield at low temperature. Shortening of SRT was found to be linked to a hydrogenotrophic methanogenic pathway, while decoupling the SRT from the HRT favoured the acetoclastic pathway for methane production.

The set of plant-wide models presented in this study provides a useful engineering tool for designing, upgrading and improving the sustainability and efficiency of wastewater treatment systems involving AD of municipal sludge. Based on the simulation results, it was found that combining sludge ozonation could impart added benefits to AD in terms of enhanced digester performance (VSS reduction and biogas production), reduced carbon footprint, and operational cost. Hence, the presented technology is more sustainable than conventional AD and provides substantial arguments for its commercialization and implementation at full-scale.

7.2 IMPLICATIONS FOR NORTH AMERICAN MARKETS

Biogas production plants for the treatment of wet-waste biomass, from wastewater treatment facilities, have witnessed an increase worldwide in several countries. Upgrading of AD installations to produce high-quality methane for use as vehicle fuel or for injection into the natural gas grid has also taken an upward trend (Börjesson & Mattiasson, 2008). In developing countries, biogas is mainly produced at small, domestic-scale level for cooking or lighting, while in developed nations, biogas developments have focussed on larger-scale, farm-based and commercial, electricity and heat biogas plants. Even among developed countries, discrepancies exist in AD trends (Fig. 7.1). Despite the significant economic and environmental benefits of generating renewable energy from organic waste streams, North America has fewer than 1,484 AD installations, and lags behind Europe with more than 2,838 AD plants (including AD plants for biosolids, municipal wastes, farms wastes, industrial solid wastes and wastewaters) (ABC, 2016; EBA, 2016). In Europe, most biomethane production from water resource recovery facilities are located in Germany (185 plants), UK (80 plants) and Sweden (61 plants) (EBA, 2016). From an environmental point of view, Europe has adopted stringent regulations limiting landfilling of organic wastes and promoting their treatment by composting or biogas production. In Northern America diversion of biomass from landfill sites is not widespread although actions have been triggered in that direction. For instance, in Canada, bans on landfilling organic wastes (including municipal biosolids) are in place in Nova Scotia and Prince Edward Island, and one is to be instated in Quebec by 2022 (Villeneuve & Dessureault, 2011), while the other provinces still permit such practice. On the other front, European governments ensure the economic viability and funding of AD installations by providing economic support with long-term subsidized power purchase agreements (PPAs). Unfortunately, this is not the case in North America. Economics (construction

and operational costs) is the key factor driving the development of AD processes and installation of biogas plants. AD projects typically earn revenues from three sources: converting biogas into electricity for sale to the grid, charging tipping fees for processing organic wastes, and selling digestate as bio-fertilizer. Operation of AD systems is highly expensive and economic viability depends on generating biogas and nutrient rich soil fertilizers. While an average AD project payback time is 5 to 7 years, some feasibility studies estimate a longer period (RWI, 2013; Scano et al., 2014; USEPA., 2015). Capital costs are high due to the equipment necessary for biogas production and purification (Lou et al., 2013; RWI, 2013). The operation and maintenance costs are also considerable at AD facilities (Moriarty, 2013). To minimize payback time, it is necessary to maximize all revenue streams of an AD project by converting biogas into electricity for sale, recovering available thermal energy, charging tipping fees for processing wastes, and selling the digestate as a bio-fertilizer (Moriarty, 2013; RWI, 2013; Scano et al., 2014; USEPA., 2015). Tipping fees alone are generally insufficient to fund an AD system.

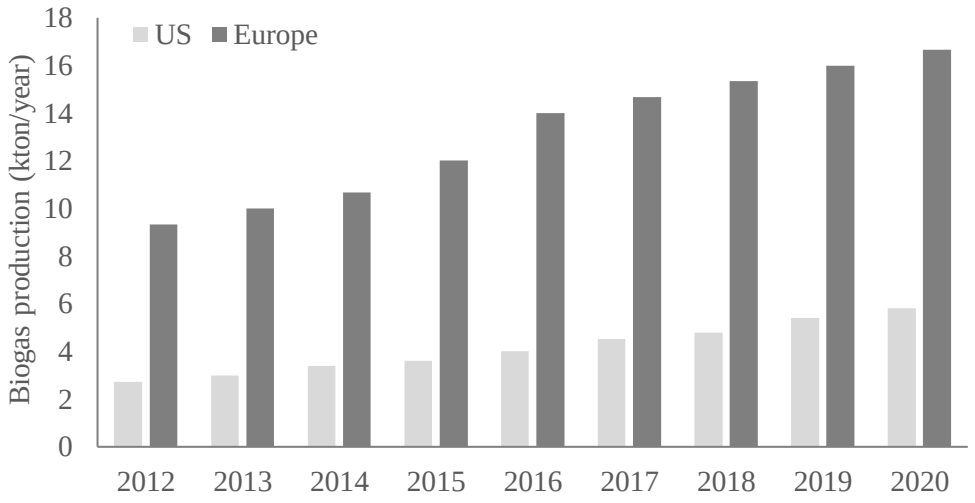

Fig 7.1. Biogas production in North America vs. Europe (ABC, 2016; EBA, 2016).

The current proposed technology holds both economic and environmental incentives over traditional AD processes. From an energy standpoint, operating AD systems at low mesophilic temperature (20 ºC) with ozonated sludge significantly reduces the energy footprints of AD installations. By operating the RAEBL facility at 20 ºC with sludge pre-treatment by ozone instead of at 35 ºC with the hydrolyzer unit, it was possible to save up to 30% of energy. This energy

conservation is significant and can be an extremely effective way of reducing energy consumption at wastewater treatment facilities. This is, in turn, translated into a reduced carbon footprint of AD installations and may constitute a more environmentally sustainable option over conventional AD at 35 ºC. From an economic perspective, operating AD units at 20 ºC with sludge ozonation reduced the operational cost by 17% in comparison with a mode of operation involving AD at 35 ºC with only thermal hydrolysis. Such enhanced attributes in conjunction with cost improvements can help to pave the way for the development of AD plants in North American jurisdictions and reduce the gap with respect to European countries, considering that economics is the main hurdle to AD developments. At the same time, it provides an opportunity for increasing the use of biomethane sectors such as transport, and act as an incentive for technology improvements and cost reductions.

7.3 SUSTAINABILITY ISSUES

The carbon budget of organic solid wastes treatment and disposal is significant considering the amount of GHG emitted. Sewage sludge is recognized as a major source of biogenic and abiogenic greenhouse gas emissions (GHG) (Sahely et al., 2006) and has been shown to emit carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Shahabadi et al., 2010). Accumulation of emitted GHGs has increased rapidly and threatens human beings as well as the environment (Kyung et al., 2015). According to the Canadian Greenhouse Gas Inventory (CGHGI, 2015), Canada's GHG emissions were 722 megatons of carbon dioxide equivalent (Mt CO_2 eq) and experienced a net decrease of 16 Mt in total emissions (2.2%) from 2005 emissions (considered as the base year for both Canada's 2020 and 2030 targets for reporting to the United Nations Framework Convention on Climate Change (UNFCCC)). Reports of Canada's GHG Emissions by IPCC sector show that wastes (solid wastes disposal, wastewater handling and wastes incineration) contributed 25 Mt (3.4%) to Canada's total emissions in 2015 and 28 Mt (3.7%) in 2005. Solid wastes disposal, which includes municipal solid wastes (MSW) and wood wastes landfills, was found to be the main GHG emitter. In 2015, solid wastes disposal accounted for 90% of wastes emissions, while biological treatment of solid wastes (composting), wastewater treatment and discharge, and incineration and open burning of wastes contributed 3.8%, 4.3% Mt and 2.2%, respectively (CBA, 2013). From the GHGs, CH_4 was found to make up 86% of the emissions from solid wastes disposal with 30 Mt CO_2 eq of CH_4 generated by MSW landfills in 2015, from which 19 Mt (or 62% of generated

emissions) were emitted to the atmosphere; the remaining 11 Mt were captured and flared at 81 landfill gas collection sites. To achieve its target as set by the Paris Climate Accord, Canada must reduce its total economy-wide emissions from 750 Mt CO_2eq (in 2005) to 523 Mt CO2eq by 2030; this would require consolidating policies and measures at the federal, provincial and territorial levels. Since predictions indicate that it would be difficult to achieve this target by the required set time, the Federal Government, has recently implemented a coordinated nation-wide carbon tax on fossil fuels, beginning at $10 per ton of CO_2eq in 2018 and rising to $50 per ton (ECCC, 2018). According to the Federal government, this measure is expected to reduce carbon pollution by 50M tons by 2022 (equivalent to taking off 12M cars off the road or closing 14 coal plants) (CBA, 2013).

Based on a survey carried out by Environment Canada in 1996, about 55% of the biosolids produced by the 50 largest WRRFs in Canada are anaerobically digested (CBA, 2013). Currently, the biogas produced from digesters is used in various ways and its usage is different from one WRRF to another. During the winter season, most WRRFs use the produced biogas in boilers and heating system for digesters while in summer the excess gas is flared. Also, for electricity production, either gas engines or micro turbines are used, while the recovered heat is used for digester and plant heating. In Canadian WRRFs, the digestate is dewatered and generally used as Class B fertilizer on agricultural land. In some locations where there is insufficient land (e.g. Durham Ontario), the dewatered biosolids are incinerated. The Canadian Biogas Association (2013) recommended that an updated assessment be done to study the potential increase in energy by utilizing biogas at WRRFs across Canada.

The currently developed low temperature AD technology involves a lower carbon footprint (by 10%) compared to conventional AD at 35 °C and can effectively aid in the decarbonization of the solid wastes handling sector. The models developed and presented in the current research indicate that sludge treatment represents the highest emitter of GHGs (~80% of the total direct and indirect GHG emission). On the one hand, ozonation pre-treatment significantly reduces the final amount of sludge produced and exiting the wastewater treatment system, thereby lowering the level of GHG associated with transport and landfilling of dewatered biosolids, and on the other, it entails a lower supply of thermal energy which aids in reducing the carbon footprint of AD systems. However, as discussed in Chapter 6, enhancing anaerobic digestion would result in a higher NH_4^+-

N concentration in the digestate, which consequently would increase the aeration demands in the activated sludge basins (Bougrier et al., 2007; Manterola et al., 2007). In order to maintain sufficient aeration, increasing blower energy would be required. Hence, this urges us to rethink the way municipal sludge is currently being digested using conventional AD processes and calls for adoption of more environmentally sustainable means for achieving equivalent or better results in terms of sludge treatment and biogas production. In addition, AD systems can represent a means to improve the generation of renewable energy from biogas to supplant fossil fuel consumption. Studies have shown that global biogas production and market are constantly expanding; however, the generation of biopower should be accelerated in view of achieving targets set by sustainability policies regarding the implementation of low carbon economies.

7.4 IMPLICATIONS FOR COLD CLIMATES

Most industrial AD installations carry excessive heating costs. In North American jurisdictions including Canada, operating anaerobic digesters in the high mesophilic temperature range (30-37 °C), especially during winter, implies a significant energy budget and cost because of the low temperature of the produced sludge resulting from aerobic treatment. Consequently, low mesophilic temperature AD represents a cost-effective strategy for wastewater treatment plant operators. According to Grant and Lin (1995), depending on the temperature of the matrix to be treated and the particular climatic conditions, it is not always practical to operate at the optimum temperature range because of the high energy requirements. Puchajda and Oleszkiewicz (2008) estimated that the operation of an anaerobic digester at 35 °C would entail an energy expenditure of about 45% of the biogas produced, in order to heat the reactor. Based on similar assumptions, we estimated that the energy expenditure could be reduced by 30% if an anaerobic digester would be operated at 20 °C. Operating at low temperature can compromise performance of anaerobic digesters such as causing solids accumulation inside the digester that reduces methanogenic activity of the sludge, eventually leading to poor mixing and digester failure due to a decrease in biogas production (Chernicharo et al., 2015). Most importantly, hydrolysis of complex organic matter becomes a limiting step at low temperatures (Daud et al., 2018). As shown in the current study, combining the AD process with sludge ozonation can effectively eliminate these limitations to significantly reduce the VSS as well as increase the methane yield of the digester. Since, digester operation at low temperature represents a more sustainable option with less energy requirements,

researches have explored strategies to boost performance of AD systems at low temperature for implementation in cold climates. Example include applying a pulse feeding protocol to increase mixing and process performance (De Vrieze et al., 2013) and use of COD balance data as a performance evaluation tool. Authors have explored COD balance linked to microbial community analyses to determine best reactor yields (Zhang et al., 2018) or as an indicator to improve energy efficiency in new operating methods (Xu et al., 2018).

From the many challenges posed by biogas technology, climatic conditions (temperature) appear to be critical for their successful implementation. Biogas production of high mesophilic and thermophilic AD is well understood. However, little research has been conducted on AD at low mesophilic and psychrophilic temperatures. What is known is that at lower temperature, longer SRTs are required to achieve similar gas production as at higher temperature (Zeeman, 1991). In winter climates, northern countries are known to face significant difficulties in maintaining the efficiency of AD installations. In some cases, supply of significant thermal energy is applied to maintain biogas production; however, this can be capital intensive for large-scale facilities. This is also true for small-scale systems where external heating is expensive (Evans et al., 2017). This is why in the latter case, other options have been considered such as underground digesters for good insulation and passive solar heating. However, even for passive solar heating there should be sufficient solar irradiation to aid in supplying thermal energy to the digester. This is not evident in countries experiencing heavy snowfalls or cold day and nocturnal temperatures as is the case for Canada. Hence, dissemination and adoption of biogas technology is limited in cold climates and their implementation requires a significant supply of thermal energy. The proposed sludge ozonation-AD hybrid technology can overcome the above described limitations to maintain AD processes in cold climates. It fits well with strategies formulated by northern countries including Canada to embark on an energy transition program to achieve sustainability. According to Government du Québec (2016) various decisions have been adopted to ensure an integrated governance of energy transition, to foster a transition to a low-carbon economy, to propose a renewed and diversified energy offer to consumers and to define a new approach to fossil fuels. The aim is to enhance energy efficiency by 15%, reduce the consumption of petroleum products by 40%, eliminate the use of coal, increase by 25% the overall renewable energy output and increase by 50% bioenergy production. The current research outcome can effectively aid in achieving these aims. As a result, a technological showcase is slowly emerging in Canada and

Quebec to identify viable projects involving the feasibility of low temperature AD of organic wastes. Recently, a Canadian research group has developed a psychrophilic AD sequencing batch reactor (PADSBR) for treating food wastes at 20 ºC (Rajagopal et al., 2017). In collaboration with Bio-Terre Systems Inc., they employed acclimated biomass at low temperature (20 ºC) to particularly degrade organic food wastes with less energy to heat up the anaerobic digester. Their results showed that methane production from food wastes was feasible at low-temperature and resulted in a specific methane yield of 0.401 ± 0.01 m3 CH_4/kg VS_{in}. Although proven successful with food wastes which contain high levels of readily degradable COD, such approach may encounter operational constraints with municipal sludge with high concentrations of non-degradable or inert substrates. Application of ozone seems to be a better choice in this case, since it has been shown to be a powerful oxidizing agent capable of improving biodegradability of biosolids containing high levels of recalcitrant COD (Chu et al., 2009). However, exploring a wide range of applications and combined solutions appears to be the best way to go about maintaining AD technologies as an integral part of the energy transition strategy in cold climate countries.

7.5 REDUNDANCY IN DESIGN SYSTEMS

It is strongly recommended that WRRFs implement redundant system design that help ensure uninterrupted operation or multi-application of a treatment for different matrices to enable efficient operation and cost saving. Even a brief disruption can cause a treatment system to reset or shut down potentially resulting in contaminants entering natural water bodies. Whether integrated into the electrical distribution system, communication network or automation process, redundant systems increase reliability and efficiency of the treatment process. The use of ozone for municipal wastewater disinfection started in the United States in the early 1970s (Rice et al., 1981) and witnessed a gradual increase in the number of facilities using this process. Its main application was for effluent disinfection and odor abatement. However, due to a change in the USEPA disinfection policy in 1976, higher capital cost, and operational issues there was a decline in the use of ozone for disinfection of treated effluent (Rice, 1999). However, since 1990s concerns about the presence of contaminants of emerging concern (CECs) such as pharmaceuticals and personal care products (PPCPs) and endocrine disrupting compounds in treated effluent and their effects on aquatic life and public health has renewed the interest in ozone treatment of municipal treated wastewater for disinfection as well as transformation of emerging contaminants (Rivera-Utrilla et al., 2013).

Studies have shown the successful removal of pharmaceuticals using advanced oxidation process such as ozone (Rivera-Utrilla et al., 2013; Ternes et al., 2003). Choi et al. (2012) showed that with over 5 mg/l of ozone oxidation, most pharmaceuticals which survived the DAF (dissolved air flotation)-MBR (membrane bioreactor) process were completely removed. These compounds, like many other CECs have been shown to be resistant to physicochemical and biological treatment at WRRFs and are therefore continuously introduced into the aquatic environment (Boleda et al., 2009; Bolong et al., 2009; Huerta-Fontela et al., 2008).

Montreal has recently called to build the largest ozonation wastewater treatment facility in the world. When completed, the ozonation centre at the J.R. Marcotte WRRF in Pointe-aux-Trembles on the eastern edge of Montreal island will have an estimated cost of $285 million and will be able to treat 2.5-7.6 million cubic metres of wastewater daily prior to discharge into the St. Lawrence River. This ozonation system is expected to eliminate 95% of bacteria (particularly *E. coli* and faecal coliforms), viruses and pharmacological drugs. In the same line, the Régie d'assainissement des eaux usées du bassin de La Prairie (RAEBL), has recently invested $18.3 million for the construction of an ozonation plant for disinfection of its effluent prior to discharge into the St. Lawrence River. The plant also houses two biogas plants which were recently constructed. Our modeling data has shown that implementing a new configuration combining sludge ozonation at low mesophilic temperature (20 ºC) at the RAEBL WRRF presents more benefits in terms of higher energy efficiency, lower carbon footprint and reduced operational cost. If such a configuration were to be implemented in the plant design, added benefits can also be gained by using the onsite ozonation installation for disinfecting the wastewater as well as pretreating its sludge in order to enable AD at low mesophilic temperature without supply of additional energy to increase the temperature to 35 ºC. This would add redundancy to the treatment system by enabling dual use of the ozone unit. This would also make sense since 30% of CECs end up in biosolids (Daughton, 2013), and a sludge pre-treatment with ozone would help mitigate their concentrations. According to the USEPA (Part 503 Rule), only biosolids meeting Class A or B requirements can be applied to agricultural lands (USEPA, 2003). Class A requirements specify a reduction of pathogen indicators such as fecal coliforms, Salmonella spp., enteric viruses and helminth ova, to essentially pathogen free biosolids while Class B requirements indicate a relatively low concentration of fecal coliform (below 2×106 MPN/g TS or CFU/g TS) with restricted land applications for grazing animals and public access and waiting periods of 1-38

months. Canadian guidelines follow similar regulations to those of USEPA (CCME, 2010). The presence of CECs in biosolids and their application to agricultural lands raises the question of whether "Class A" biosolids should be redefined. It would be prudent to recognize the potential risks of chronic exposures to CECs present in biosolids and the health effects associated with the consumption of crops fertilized with such type of sewage wastes. Hence, pre-treatment of sludge by ozonation and AD treatment can improve removal of CECs from biosolids and enhance its status to "Class A+" for instance thereby contributing to its marketing campaign as a beneficial, cheap and risk-free fertilizer. A recent study by Wu et al. (2012) has reported the transfer of PPCPs such as carbamazepine, diphenhydramine and triclocarban from biosolids amended soils into tissues of crops such as tomato, pepper, collard, lettuce and radish. Is there anything we can do differently in order to eliminate the problem associated with sewage sludge? One solution might effectively be implementing a redundant system involving sludge ozonation to limit the biosolid impacts on nature and public health especially with the current limited regulations with respect to CECs. Our proposed approach of combining sludge ozonation with AD treatment may well help to eliminate CECs and open up avenues for imposing regulatory restrictions on biosolids applied to agricultural lands.

7.6 FINAL WORDS

This research outlines a novel approach for treating municipal biosolids by AD combining ozonation at low mesophilic temperature (20 ºC). Compared to conventional standalone AD treatment of biosolids at 35 ºC, this configuration is more efficient in terms of performance and is environmentally and economically more sustainable. In the past, the answer to the question of how to use biogas from AD of wastes was either to produce hot water or electricity. Today, the answer is much more complex and integrates aspects such as sludge reduction, energy transition, carbon footprint reduction and climate change. The proposed technology enables energy recovery from municipal wastes at low energy expenditure and this can have tremendous impacts on the ability of municipal, provincial and federal administrations to meet their objectives in terms of biosolids management, shift to renewable energy and establishing a low carbon economy. The primary concern of WRRF operators is to reduce the production of excess sludge considering that the cost of biosolids disposal can be as high as 60% of the total plant operational cost (Liu, 2003). The proposed sludge ozonation-AD hybrid technology at low temperature can reduce sludge

production by 20% and hence, represents considerable savings for municipalities and tax payers. In addition, landfilling and incineration of biosolids have been banned in Nova Scotia and Prince Edward Island, and one is to be instated in Quebec by 2022 (CCME, 2010; Villeneuve & Dessureault, 2011). It will not be long for such measure to reach out other provinces as well. By reducing the amount of biosolids through ozonation and AD, WRRFs will be in a better position to meet the new regulatory requirements in terms of biosolids disposal and diversion from landfill and incineration sites.

In addition, the proposed technology is likely to increase the energy recovery and energy efficiency of WRRFs. Considering that the temperature of wastewater is below 12 ºC during the cold season, which spans for 4-6 months yearly, the produced methane can be used to heat up the WAS to 20 ºC. Also, an enhanced biogas production is expected without the need to increase in the size of the digester. Based on a report by the Canada Biogas Association (CBA, 2013), available technologies would allow WRRFs to produce up to 7% of the renewable biogas that could be potentially produced in Canada. Currently, the potential to produce biogas and use it as a renewable source of energy have not been fully realized in Canada. The new process can enable WRRFs operators to maintain or increase their biogas production without excessive energy expenditure or increase in their digester capacity and facilitate the integration of waste biomass as a sustainable source of energy. Bioenergy accounts for approximately 6% of Canada's total energy supply (NRC, 2018), and government's vision is to increase the share of sustainable bioenergy in Canada's energy mix and advance Canada's bioenergy supply through development of new technologies. This is in also in line with Canada's requirement to fulfill the Paris Accord at the COP21 Conference in fall 2015 to reduce its total economy-wide GHG emissions from 750 Mt CO2eq (baseline of 2005) to 523 Mt CO2eq by 2030. The proposed technology forms part of low carbon economy initiatives along with cap and trade, carbon fixation and emission targets, and provides impetus for implementation of AD processes to treat biosolids rather than having recourse to landfilling or incineration. Thus, by improving methane yields from the AD of WAS, the new pre-ozonation process could significantly contribute to improving the sustainability of Canadian public infrastructures by reducing economic burden, reducing greenhouse gas emissions, and reducing biosolids disposal. Finally, the savings on the biosolids disposal costs and the additional capacity to produce biogas make it even more attractive for WRRF to develop the biogas production potential by investing in AD of WAS, which provides an additional investment incentive for WRRF operators. This

transdisciplinary research provides insights to environmental engineers, plant operators and modelers, for the conception, design and operation of AD installations with new configurations aimed at promoting the use of bioenergy derived from organic wastes.

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