TWO-PHASE ANAEROBIC DIGESTION TO REDUCE
THE NO_x EMISSION POTENTIAL OF BIOGAS

A Thesis

presented to

the Faculty of California Polytechnic State University,

San Luis Obispo

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Civil and Environmental Engineering

by

Nathaniel Manuel Olivas

December 2015

© 2015

NATHANIEL MANUEL OLIVAS

ALL RIGHTS RESERVED

COMMITTEE MEMBERSHIP

TITLE: Two-Phase Anaerobic Digestion to Reduce the
NOx Emission Potential of Biogas

AUTHOR: Nathaniel Manuel Olivas

DATE SUBMITTED: December 2015

COMMITTEE CHAIR: Tryg Lundquist, Ph.D., P.E.
Associate Professor of Environmental Engineering

COMMITTEE MEMBER: Yarrow Nelson, Ph.D.
Department Chair of Civil and Environmental
Engineering

COMMITTEE MEMBER: Gregory Schwartz, Ph.D.
Assistant Professor of BioResource and
Agricultural Engineering

ABSTRACT

Two-Phase Anaerobic Digestion to Reduce the NO_x Emission Potential of Biogas

Nathaniel Manuel Olivas

Anaerobic digestion can be used to decrease the mass of organic wastes to be disposed of while producing useful biogas (CH₄ and CO₂) for heat or power production, but in air basins with strict emissions limits, biogas combustion is difficult to implement due to the high costs of controlling NO_x emissions. NO_x production can be minimized by blending H₂ gas with CH₄ at a volume ratio of 15:85 H₂:CH₄, which allows burning at ultra-lean air-to-fuel ratios. For biogas systems, a potential low-cost NO_x control strategy is to produce H₂-CH₄ mixtures through two-phase anaerobic digestion, where two digester tanks are operated in series, with the first one producing a majority H₂ and the second CH₄. The resulting mixture of H₂, CH₄, and CO₂ should combust with low NO_x emissions. Furthermore, in theory, if the biogas from the second-phase is sparged through the first-phase, H₂ would be stripped from the first-phase liquid medium, and H₂ production would be more thermodynamically favored, possibly increasing H₂ production.

Laboratory experiments were used to determine the optimal conditions to generate biogas with a 15:85 H₂:CH₄ ratio using two phase digestion with glucose as the substrate. Specifically, the objectives of this thesis were to (1) determine the optimal conditions for operating the first-phase to produce H₂, (2)

determine the sparging rate required to achieve 15:85 H₂:CH₄ in the biogas, and (3) operate the first and second-phases together with second-phase biogas being sparged through first-phase medium to achieve 15:85 H₂:CH₄. The results from each of these objectives are described below.

(1) The optimal conditions for H₂ production in the first-phase were an organic loading rate of 22.9 g COD/L-day (chemical oxygen demand) and a hydraulic residence time of 12 hours. The resulting pH in the first-phase was 6.11 when operated under these conditions. Optimized hydrogen production in the first phase resulted in the generation of 1.02 ± 0.13 L H₂/L_{digester}-day, which can also be expressed as 0.61 ± 0.10 mol H₂/mol glucose_{consumed}, 0.42 ± 0.06 mol H₂/mol glucose_{introduced}, 1.06 ± 0.16 mol H₂/mol COD_{destroyed}, and 0.06 ± 0.01 mol H₂/mol COD_{introduced}.

(2) Initial sparging experiments were conducted using nitrogen (N₂) to represent second-phase biogas. The rates tested ranged from 1- 30 L N₂/L_{first-phase digester}-hr. A 1.1 L gas/L-hr sparging rate was projected to result in a 15:85 H₂:CH₄ ratio. The projection was made using a power regression model ($R^2 = 0.99$) of sparging rate vs. hydrogen content results, assuming the sparged N₂ was replaced with typical biogas (60% CH₄ and 40% CO₂).

(3) When both phases were integrated, the second-phase produced enough gas to sparge at only 0.28 L gas/L_{first-phase digester}-hr, which was far less than the optimal 1.1 L gas/L_{first-phase digester}-hr sparging rate. A non-optimal H₂:CH₄ ratio of

15:12 was obtained at the 0.28 L gas/L-hr sparging rate. Insufficient CH₄ was generated due to the low organic loading provided to the second-phase.

Although the 1.1 L gas/L-hr sparging rate was not tested in an integrated system, the results obtained from the 0.28 L gas/L-hr sparging rate differed from what was predicted by the nitrogen sparging model by only 14%. Therefore, the model was fairly accurate (at least at a low flow rate of 0.28 L gas/L-hr) and could still be valid for the predicted optimal flow rate of 1.1 L gas/L-hr.

For future two-phase digestion studies, biogas production from the second-phase can be increased by adding more substrate to the second-phase or by using fixed-film digesters to possibly increase the number density of methanogens. It is also recommended to digest practical waste feedstocks, and possibly digest different feedstocks in the first and second-phases. Also, the effects of carbon dioxide on the combustion characteristics and NO_x emissions of hydrogen-methane mixtures in biogas need to be researched.

ACKNOWLEDGMENTS

I would like to acknowledge the following people and organizations for helping me achieve my research goals and supporting me while I attended Cal Poly:

- Ruth Spierling: It was great working with you. Thank you for being an excellent, dedicated, and patient supervisor. I appreciate all the things you've helped me with on this project and as a student, and for letting me struggle and fail at times. Your supervision has made me become a better engineer and I will be forever grateful for that.
- Tryg Lundquist: Thank you for choosing me to work on this project, teaching me the ways of wastewater engineering, providing me guidance, and editing my thesis.
- John Benemann: I appreciate the insight you provided to better improving my research.
- San Luis Obispo Wastewater Reclamation Facility Staff: Thank you for letting me grab sludge every few weeks and for still letting me visit even after I dropped a sample bottle into one of the digester standpipes!
- California Energy Commission (CEC) & Agricultural Research Institute (ARI): Thank you for the funding which supported my thesis project.
- Yarrow Nelson & Gregory Schwartz: Thank you for being on my thesis committee and providing additional feedback on my project.
- Shelley Blackwell: I am grateful for your help in managing my undergraduate research assistants, taking care of purchasing items for my project, and ensuring the labs were well-stocked.
- Tracy Thatcher: Thank you for letting me borrow your air flow calibration meter and being an excellent advisor and teacher.

- Neal Adler, Sean Thomson, & Kyle Fooks: Thank you for letting me work on your thesis projects and giving me valuable research experience as an ENVE 400 student.
- Chris Pittner, Erik Hoffnagle, & Hayley Baker: Thank you for working on the more intricate parts of my project, whether it was operating and maintaining the digester system independently, constructing gas meters, or conducting lab tests. It was great working with all of you.
- All ENVE 400/405 Students: I appreciate all the work you put into helping me carry out my project. Without you this research would not have been possible. In the words of NBA superstar Kevin Durant: “You da real MVP.”
- My Fellow Graduate Students: It was great working with you in the labs and going through the thesis experience together.
- Amy Sinclair, Kay Kibbe, and Xi Shen: Thanks for ensuring the school vehicles were kept in good-condition and available when I needed to go to the sewage plant.
- Cal Poly San Luis Obispo: Thank you for providing me with a quality education and the knowledge to become a good engineer.
- Merle Fry: Thanks for putting up with the Breeze Lab. Although we did not know each other too well, I know you occasionally saw sludge on the floors or smelled some funky odors. I greatly appreciate you ensuring the paper-towel and soap dispensers were always full and dealing with our waste.
- My Parents & Steve: Thank you for supporting me in life and my education. I would have never gotten to this point without you guys.
- Marissa Carranza: Thank you for hanging in there through the ups-and-downs of my project, and for always being someone I could bounce ideas off of. I am glad to have had you as my partner-in-crime during this project.

TABLE OF CONTENTS

	Page
LIST OF TABLES	xiii
LIST OF FIGURES	xvi
LIST OF EQUATIONS.....	xx
CHAPTER 1 : INTRODUCTION.....	1
1.1 Thesis Outline.....	3
CHAPTER 2 : BACKGROUND	4
2.1 Significance and History	4
2.1.1 Greenhouse Gases and How Dairy Farms Contribute to Emissions ...	4
2.1.2 Reducing GHG Emissions with Controlled Anaerobic Digestion.....	5
2.1.3 NO _x Emission Problems from Combusting Biogas.....	6
2.1.4 Hydrogen Addition to Reduce NO _x Emissions from Farm Digesters ...	7
2.1.5 Hydrogen Production, Storage, and Transportation Issues	9
2.1.6 Fermentative Hydrogen Production	9
2.1.7 Two-Phase Anaerobic Digestion as the Solution.....	10
2.2 Anaerobic Digestion Fundamentals.....	11
2.3 Two-Phase Digestion.....	14
2.3.1 Methods to Produce Hydrogen in the First-Phase	14
2.4 Previous Studies on Fermentative Hydrogen Production	16
2.4.1 Optimal Operating Conditions.....	18
2.4.2 Issue of Low Molar Hydrogen Yields	21
2.4.3 Methods to Increase Hydrogen Yields	23
2.5 Project Goals	24
2.5.1 First-Phase Optimization	25
2.5.2 Nitrogen Sparging.....	25
2.5.3 Integrated First and Second-Phase Operation	25
CHAPTER 3 : MATERIALS & METHODS.....	27
3.1 Apparatus & Experiment Setup	27
3.1.1 Design of Bench-Top Anaerobic Digesters.....	30

3.1.2 Gas Meter Design.....	33
3.1.3 Leak Testing of Digesters	35
3.1.4 Peristaltic Pump Setup	36
3.1.5 Feedstock Reservoir Design.....	37
3.1.6 Nitrogen Sparging Experiment Apparatus	39
3.1.7 Feedstock Ingredients & pH Control	40
3.1.8 Digester Inoculum Source	42
3.1.9 Serum Bottle Experiment Setup	43
3.2 Analytical Test Methods.....	44
3.2.1 pH & Alkalinity.....	45
3.2.2 Biogas Composition.....	45
3.2.3 Chemical Oxygen Demand	46
3.2.4 Total and Volatile Suspended Solids	46
3.2.5 Glucose Concentration	47
3.2.6 Quality Control Procedures.....	48
3.3 Operations & Maintenance	49
3.3.1 Experiment Startup Procedure	49
3.3.2 Daily Maintenance Tasks.....	50
3.3.3 Steady State Data Collection	52
3.3.4 Experiment Shutdown Procedure	53
3.3.5 Steady-State Period Verification	53
3.3.6 Hydrogen Yield Analysis.....	54
3.4 3D Surface Modeling Procedures.....	55
CHAPTER 4 : FIRST-PHASE OPTIMIZATION EXPERIMENTS.....	57
4.1 Issues with Initial First-Phase Experiments	57
4.2 Serum Bottle Experiment.....	59
4.2.1 Experimental Procedures	59
4.2.2 Results.....	62
4.2.3 Discussion	64
4.2.4 Adjustments to Future Experiments	66

4.3 Organic Loading Rate (OLR) Testing Experiment	67
4.3.1 Organic Loading Rate Correction	67
4.3.2 Graphing of OLRs within pH 5.0 – 6.5	69
4.3.3 Volumetric Hydrogen Yields & Hydrogen Composition	69
4.3.4 Glucose Consumption	70
4.3.5 Glucose-Consumed & Glucose-Introduced Hydrogen Yields	72
4.3.6 COD Destruction.....	74
4.3.7 COD-Introduced & COD-Destroyed Hydrogen Yields.....	75
4.3.8 Chemical Oxygen Demand (COD) Balance.....	77
4.3.9 Discussion	78
4.3.10 Ideal Operating OLR.....	81
4.4 Hydraulic Residence Time (HRT) Experiment	82
4.4.1 Experimental Procedures	82
4.4.2 Volumetric Hydrogen Yields & Hydrogen Composition	83
4.4.3 Glucose Consumption	84
4.4.4 Glucose-Consumed & Glucose-Introduced Hydrogen Yields	86
4.4.5 COD Destruction.....	87
4.4.6 COD-Introduced & COD-Destroyed Hydrogen Yields.....	88
4.4.7 Chemical Oxygen Demand (COD) Balance.....	89
4.4.8 Discussion	91
4.4.9 Ideal Operating HRT.....	92
4.5 3D Surface Model Summary.....	93
CHAPTER 5 : NITROGEN SPARGING & INTEGRATED FIRST AND SECOND-PHASE EXPERIMENTS	98
5.1 Nitrogen Sparging Experiment.....	98
5.1.1 Experimental Procedures	98
5.1.2 Organic Loading Rate and pH	99
5.1.3 Volumetric Hydrogen Yields	100
5.1.4 Glucose Consumption	101
5.1.5 Glucose-Consumed & Glucose-Introduced Hydrogen Yields	101

5.1.6 COD Destruction.....	102
5.1.7 COD-Destruction & COD-Introduced Hydrogen Yields.....	102
5.1.8 Chemical Oxygen Demand (COD) Balance.....	104
5.1.9 Mixed Gas Composition.....	105
5.1.10 Hydrogen Composition Prediction Model.....	105
5.1.11 Discussion	106
5.2 Integrated First and Second-Phase Experiment	107
5.2.1 Experimental Procedures	107
5.2.2 Results.....	110
5.2.3 Second-Phase COD Balance	111
5.2.4 Discussion	112
CHAPTER 6 : CONCLUSIONS	114
6.1 Experimental Conclusions	114
6.2 Research Limitations	115
6.3 Recommendations.....	116
6.3.1 Attached Growth Research.....	116
6.3.2 Practical Substrates for Hydrogen Production	118
6.3.3 Digestion of Different Substrates in the First-Phase	118
6.3.4 Fixed Film Second-Phase Digesters.....	119
6.3.5 Digester Volume Ratio Optimization	119
6.3.6 Methane Reformation of Biogas to Hydrogen.....	120
6.3.7 Effect of CO ₂ on Combustion Characteristics of H ₂ /CH ₄ Blends	120
6.3.8 Effect of Mixing on H ₂ Production	121
6.3.9 Lactic Acid Bacteria	122
6.3.10 Volatile Fatty Acid Testing	122
6.3.11 Testing Realistic Sparging Rates.....	123
REFERENCES	124
APPENDICES	
Appendix A – Second-Phase Gas Production Rate Calculations	130

LIST OF TABLES

Table	Page
Table 2-1: Effect of emission controls on NO _x emissions from IC engines (Wilson, 2012)	8
Table 2-2: Summary of ideal operating conditions for fermentative hydrogen production from the studies discussed in Section 2.4.1	21
Table 2-3: Gibbs Free Energy of Formation (ΔG°) and theoretical molar H ₂ yields for the various pathways of glucose degradation in fermentative hydrogen production (Karakashev & Angelidaki, 2011)	22
Table 3-1: Descriptions of the types of H ₂ yields analyzed for all 1 st -phase optimization	54
Table 4-1: Setup used for the Serum Bottle Experiment to aid in determining which feedstock nutrient solution was ideal, and to troubleshoot why the initial first-phase experiments had low yields. Each serum bottle (165-mL capacity) had a total of 100-mL liquid volume.	61
Table 4-2: pH, Alkalinity, and H ₂ production results for the Serum Bottle Experiment. This experiment lasted eight days..	63
Table 4-3: Feedstock glucose concentrations, COD concentrations, and calculated GLRs for all target GLRs and pHs tested. The feedstock glucose concentration expected based on COD measurements and the organic loading rate (OLR) in terms of COD were also calculated.	68
Table 4-4: Volumetric H ₂ yields, and H ₂ composition measured for all organic loading rates and pHs tested at a constant 12-hr hydraulic residence time (HRT).	70
Table 4-5: Influent (feedstock) glucose, effluent glucose, glucose consumed, and % glucose consumption at all organic loading rates (OLRs) tested at a constant 12-hr hydraulic residence time (HRT).	71
Table 4-6: H ₂ yields on a glucose-consumed and glucose-introduced basis for all organic loading rates (OLRs) and pHs tested at a constant 12-hr hydraulic residence time (HRT).	73

Table 4-7: Influent (feedstock) COD, effluent COD, and % COD destruction at all organic loading rates (OLRs) tested at a constant 12-hr hydraulic residence time (HRT).....	74
Table 4-8: H ₂ yields on a COD-destroyed and COD-introduced basis for all organic loading rates (OLRs) and pHs tested at a constant 12-hr hydraulic residence time (HRT).	76
Table 4-9: COD Balance for all organic loading rates (OLRs) tested.	77
Table 4-10: H ₂ yield summary for the ideal OLR of 22.9 g COD/L-day.....	82
Table 4-11: Summary of hydraulic residence times (HRTs) and steady-state pHs tested for the HRT experiment. All HRTs were operated at a constant organic loading rate (OLR) of 22.9 g COD/L-day.....	83
Table 4-12: Volumetric H ₂ yields, and H ₂ composition measured for all hydraulic residence times (HRTs) and pHs tested in the HRT experiment (all operated at a target organic loading rate (OLR) of 22.9 g COD/L-day).....	84
Table 4-13: Influent (feedstock) glucose, effluent glucose, glucose consumed, and % glucose consumption at all hydraulic residence times (HRTs) and pHs tested at a target organic loading rate (OLR) of 22.9 g COD/L-day.....	85
Table 4-14: H ₂ yields on a glucose-consumed and glucose-introduced basis for all hydraulic residence times (HRTs) and pHs tested.....	87
Table 4-15: Influent (feedstock) COD, effluent COD, and % COD destruction at all hydraulic residence times (HRTs) tested	88
Table 4-16: H ₂ yields on a COD-destroyed and COD-introduced basis for all hydraulic residence times (HRTs) and pHs tested.....	89
Table 4-17: COD Balance for all HRTs tested. Percent (%) recovery was calculated as Effluent COD +H ₂ COD Calculated/Influent COD×100%.....	90
Table 4-18: H ₂ yield summary for the ideal HRT of 12 hrs compared to other HRTs tested (within pH 5.0 – 6.5).....	92

Table 4-19: Maximum theoretical H ₂ yield values predicted by the 3D surface models and their corresponding operating conditions.....	97
Table 5-1: Organic loading rates (OLRs) and pHs at all sparging rates tested. The target OLR was 22.9 g COD/L-day.....	100
Table 5-2: Influent (feedstock) glucose, effluent glucose, glucose consumed, and % glucose consumption at all sparging rates tested.....	101
Table 5-3: Influent (feedstock) COD, effluent COD, and % COD destruction at all sparging rates tested.....	102
Table 5-4: COD Balance for all sparging rates tested.	104
Table 5-5: Measured H ₂ , CO ₂ , and N ₂ biogas compositions at all sparging rates tested. Estimated mixed gas compositions and H ₂ :CH ₄ ratios are also shown.	105
Table 5-6: Data summary for the first and second-phases in the integrated experiment.....	111

LIST OF FIGURES

Figure	Page
<p>Figure 2-1: Simplified overall proposed two-phase digestion system process using dairy manure as a potential feedstock. Hydrogen and methane are generated, respectively, in the 1st and second-phases by manipulating the average amount of time bacteria spend in each digester (HRT: Hydraulic Residence Time).</p>	11
<p>Figure 2-2: The four general steps of anaerobic digestion. Overall, organic matter is converted to the end products CH₄, CO₂, and nutrient-rich digestate (remaining matter after anaerobic digestion). Image from (Cooke, 2014).....</p>	12
<p>Figure 3-1: System used for conducting all first-phase and N₂ sparging experiments. The digester bottles were wrapped with heating pads covered with aluminum insulation.</p>	28
<p>Figure 3-2: Flow schematic for the first-phase digestion experiments. This particular setup was used for OLR experiments. For HRT experiments, pump tubing was configured differently (described in Section 3.1.1). Each component of the system is described in further detail in Sections 3.1.1 – 3.1.5.....</p>	29
<p>Figure 3-3: Schematic of a first-phase (H₂) anaerobic digester. The vessel rested on a stir plate and was wrapped in an electric heating pad. The protuberance shown on the top of the vessel represents the threaded cap for the bottle, which was sealed with Teflon tape to prevent gas leaks.</p>	31
<p>Figure 3-4: Schematic of the biogas flow. Gas produced in the digesters was directed through 6.4-mm ID tubing to a custom-built tipping gas meter. A 1-L Tedlar bag was used to accommodate changes in the liquid volume of the digester due to uneven pumping and slight temperature swings.....</p>	33
<p>Figure 3-5: Schematic of gas meters used for measuring gas production from the anaerobic digesters (Fresco, 2015). Note: The dimensions used for the gas meter in this drawing are a few centimeters larger than the gas meters used for this project.</p>	34

Figure 3-6: Schematic of a feedstock reservoir. The reservoirs were 20-L, continuously-mixed, and kept inside a refrigerator at 4°C.	39
Figure 3-7: Experimental apparatus used for measuring biogas production from serum bottles (Hill, 2014). The serum bottles in this figure are from a different study using algae.....	44
Figure 4-1: Cumulative hydrogen produced per for each serum bottle set. Note: Only Set 8 and Set 7 produced significant amounts of hydrogen in this experiment.....	64
Figure 4-2: Volumetric H ₂ yields measured for all organic loading rates (OLRs) tested at a constant hydraulic residence time (HRT) of 12 hrs. Only OLRs operated between pH 5.0 – 6.5 are graphed.....	70
Figure 4-3: Glucose consumption for all organic loading rates (OLRs) tested between pH 5.0 – 6.5.	72
Figure 4-4: H ₂ yields on a glucose-consumed and glucose-introduced basis at all organic loading rates (OLRs) tested at a constant hydraulic residence time (HRT) of 12 hrs. Only OLRs operated between pH 5.0 – 6.5 are graphed.	74
Figure 4-5: H ₂ yields on a COD-destroyed and COD-introduced basis at all organic loading rates (OLRs) tested at a constant hydraulic residence time (HRT) of 12 hrs. Only OLRs operated between pH 5.0 – 6.5 are graphed.	76
Figure 4-6: COD balance at all organic loading rates (OLRs) and pHs tested....	78
Figure 4-7: Volumetric H ₂ yields measured for all hydraulic residence times (HRTs) tested at a target organic loading rate (OLR) of 22.9 g COD/L-day and between pH 5.0 – 6.5.....	84
Figure 4-8: Glucose consumption at all hydraulic residence times (HRTs) tested between pH 5.0 and 6.5.....	85
Figure 4-9: H ₂ yields on a glucose-consumed and glucose-introduced basis at all hydraulic residence times (HRTs) tested. Only HRTs operated between pH 5.0 – 6.5 are graphed.....	87

Figure 4-10: H ₂ yields on a COD-destroyed and COD-introduced basis at all hydraulic residence times (HRTs) tested. Only HRTs operated between pH 5.0 – 6.5 are graphed.....	89
Figure 4-11: COD balance at all hydraulic residence times (HRTs) tested.....	90
Figure 4-12: Volumetric H ₂ Yield vs. HRT vs. OLR (within pH 5.0 – 6.5). This surface model predicts a maximum volumetric H ₂ yield of 1.02 L H ₂ /L-day at a 13.0-hr HRT and an OLR of 23.2 g COD/L-day.....	94
Figure 4-13: Glucose-consumed H ₂ Yield vs. HRT vs. OLR (within pH 5.0 – 6.5). This surface model predicts a maximum glucose-consumed H ₂ yield of 1.20 mol H ₂ /mol glucose _{consumed} at a 19.6-hr HRT and an OLR of 26.1 g COD/L-day.....	94
Figure 4-14: Glucose-introduced H ₂ Yield vs. HRT vs. OLR (within pH 5.0 – 6.5). This surface model predicts a maximum glucose-introduced H ₂ yield of 0.48 mol H ₂ /mol glucose _{introduced} at a 15.6-hr HRT and an OLR of 20.3 g COD/L-day.....	95
Figure 4-15: COD-destroyed H ₂ Yield vs. HRT vs. OLR (within pH 5.0 – 6.5). This surface model predicts a maximum COD-destroyed H ₂ yield of 1.08 mol H ₂ /mol COD _{destroyed} at an 11.6-hr HRT and an OLR of 25.2 g COD/L-day.....	95
Figure 4-16: COD-introduced H ₂ Yield vs. HRT vs. OLR (within pH 5.0 – 6.5). This surface model predicts a maximum COD-introduced H ₂ yield of 0.06 mol H ₂ /mol COD _{introduced} at a 14.0-hr HRT and an OLR of 19.5 g COD/L-day.....	96
Figure 5-1: Volumetric H ₂ yields measured at all sparging rates tested.	100
Figure 5-2: H ₂ yields on a glucose-consumed and glucose-introduced basis, and percent glucose consumed (for reference) at all sparging rates tested	102
Figure 5-3: H ₂ yields on a COD-destroyed and COD-introduced basis at all sparging rates tested.	103
Figure 5-4: COD balance at all sparging rates tested.....	104
Figure 5-5: Actual sparging rate vs. measured %H ₂ for the N ₂ sparging experiment.....	106

Figure 5-6: Schematic of the integrated first and second-phase experiment....	109
Figure 5-7: COD Balance for the second-phase digesters	112
Figure 5-8: Measured and predicted %H ₂ at a sparging rate of 0.28 L gas/L-hr.	113
Figure 6-1: Attached growth occurred on the tubing in the inside of the digesters and the inner walls.	117

LIST OF EQUATIONS

Equation	Page
Equation 2-1: Acetate Formation.....	13
Equation 2-2: Butyrate Formation.....	13
Equation 2-3: CH ₄ Formation Pathway 1	13
Equation 2-4: CH ₄ Formation Pathway 2	14
Equation 2-5: CH ₄ Formation Pathway 3	14
Equation 2-6: Glucose Loading Rate (GLR) Equation	19
Equation 3-1: Gas Meter Calculation.....	34
Equation 3-2: Hourly Pumping Volume Calculation	37
Equation 3-3: pK _a Equation	41
Equation 3-4: GLR Correction Calculation.....	47
Equation 3-5: Split Percent Error Formula	48
Equation 3-6: Spike Percent Recovery Formula.....	48

CHAPTER 1: INTRODUCTION

Anaerobic digestion is an appealing method for managing and treating biodegradable wastes because it converts organic matter into potentially useful biogas, primarily of methane (CH_4) and carbon dioxide (CO_2) (CEC, 2015). While CH_4 and CO_2 are both greenhouse gases (GHGs), if the biogas is collected, it can be combusted to generate electricity and heat (Bracmort, 2010), potentially offsetting fossil fuel use (DOE, 2013).

Most dairy farms in California use anaerobic, open lagoons to store and treat cow waste (Spierling et al., 2009), but many do not currently collect biogas for combustion (USEPA, 2010, 2015). Instead, the waste is allowed to anaerobically ferment, releasing CH_4 and CO_2 into the atmosphere (Bracmort, 2010). One reason farmers have been deterred from collecting biogas is the high capital and maintenance costs associated with using air pollution control equipment to control the nitrous oxide (NO_x) emissions that result from biogas combustion (ESA, 2011).

With increasing regulations enacted to reduce GHG emissions in California, such as the Global Warming Solution Act (AB32) and Executive Order B-30-15, the State government is interested in reducing GHG emissions from many types of sources (CARB, 2015). Dairy farms are candidates for GHG reductions because open lagoons emit CH_4 and CO_2 (Bracmort, 2010).

Controlled anaerobic digestion, where biogas is collected and burned instead of allowed to escape into the atmosphere (Metcalf & Eddy, 2013), is an increasingly

appealing option for dairy farms in California because it can reduce GHG emissions significantly (Bracmort, 2010). However, most dairies in California are in the San Joaquin Valley, which is a severe non-attainment area for ozone (CalEPA, 2011). Thus, finding a cost-effective way to reduce NO_x emissions from biogas combustion is an issue that needs to be resolved before anaerobic digestion becomes more widely used for California dairies.

A potential NO_x emissions control strategy that might be financially feasible is to enrich the biogas with hydrogen (Kornbluth et al., 2012). Studies have shown that hydrogen-methane mixtures at a ratio of approximately 15% H₂ to 85% CH₄ by volume (henceforth referred to as 15:85 H₂:CH₄), burn with significantly lower NO_x emissions when combusted at ultra lean air-to-fuel ratios (Choudhuri & Gollahalli, 2000; Collier, Hoekstra, Mulligan, Jones, & Hahn, 1996; Shrestha & Karim, 1999; Sierens, 1998). This strategy is appealing since it could potentially eliminate or reduce the need for costly post-combustion NO_x control devices, such as catalytic converters.

One way H₂-CH₄ mixtures can be produced is by manipulating the anaerobic digestion process to produce an excess of H₂ in addition to CH₄ and CO₂. Hydrogen is produced during anaerobic digestion by particular groups of bacteria, but the hydrogen is typically consumed by methanogenic bacteria, to produce CH₄ (WtERT, 2009). If the hydrogen-formers can be separated from the methanogens in a separate digester (or reactor), while another digester includes both groups of bacteria, it is possible to have both hydrogen and methane as end-products (Cooney et al., 2007). This method of generating H₂-CH₄ mixtures

is known as two-phase anaerobic digestion (Cooney et al., 2007). The term “two-phase digestion” is also commonly used to describe digester reactors operated in series but without excess H_2 production (Metcalf & Eddy, 2013), but in the present work it will be used to mean a H_2 -producing phase followed by a CH_4 -producing phase.

1.1 Thesis Outline

This thesis project was an attempt to achieve the 15:85 $H_2:CH_4$ ratio using a laboratory scale, two-phase digestion system fed glucose. Real waste substrates were not used because this was a proof-of-concept project, and it was easier to control the organic loading into the system with glucose as the substrate.

The remaining chapters in this thesis cover this project’s scientific background and goals, the system design and setup, testing methods, and experimental procedures. Results from all experiments are discussed, and conclusions and recommendations for future research are made from these results.

CHAPTER 2: BACKGROUND

In this chapter, the history and backdrop for this project are discussed in more detail. Also, the fundamentals of anaerobic digestion, two-phase digestion, fermentative hydrogen production, and research goals of this project are described.

2.1 Significance and History

The overall significance, background, and events that led to the development of this project, which were briefly summarized in Chapter 1, are discussed further in this section.

2.1.1 Greenhouse Gases and How Dairy Farms Contribute to Emissions

Since the mid 20th-century, greenhouse gases (GHGs) have been a major contributor to climate change (USEPA, 2014a). CH₄ and CO₂ are the most commonly emitted GHGs in the U.S, with CH₄ having 21 times more global warming potential than CO₂ over a 100-year period (USEPA, 2014b).

In 2012, 36% of anthropogenic CH₄ emissions in the United States came from agricultural sources (The White House, 2014). Besides enteric fermentation (CH₄ emissions by livestock), manure is the second leading source of CH₄ emissions in the agriculture sector (USEPA, 2014b). CH₄ emitted to the atmosphere from the biological breakdown of manure is a lost opportunity to combust the gas for heat and electricity and a significant contributor to climate change.

Uncontrolled CH₄ emissions are commonplace at dairy farms using lagoons to store manure (Bracmort, 2010). In the Central Valley of California, where approximately 1,500 dairy farms are located, most animals are housed in barns and corrals (Pettygrove, Putnam, & Meyer, 2003). Roughly two-thirds of these farms flush water through the alleys to remove cow waste (Krich et al., 2005). The used flush water is usually stored in an anaerobic lagoon (Spierling et al., 2009). Waste reduction is achieved as the manure undergoes anaerobic respiration in the lagoon, but the manure is partly converted to biogas which is released into the atmosphere. Waste lagoons are also exposed to the air and subject to low temperatures, making them inefficient at degrading waste and a source of VOC emissions (odor) (Krich et al., 2005).

With greenhouse gas regulations, the California state government is increasingly interested in reducing GHG emissions, particularly from stationary sources such as dairy waste lagoons. The Global Warming Solutions Act (AB32) and Executive Order B-30-15 require Californians to reduce their GHG emissions (CARB, 2015), leading to increased urgency to divert GHGs from dairy and animal waste and to generate carbon offset credits as a potential revenue source.

2.1.2 Reducing GHG Emissions with Controlled Anaerobic Digestion

A solution for reducing both waste and GHG emissions is controlled anaerobic digestion. In a controlled environment, better conditions for anaerobic respiration are provided, resulting in accelerated waste reduction. Additionally, biogas is captured instead of released into the atmosphere. The biogas can be used to

produce electricity which may further reduce carbon emissions by offsetting fossil fuel consumption.

Controlled anaerobic digestion is an appealing option because it is relatively inexpensive, easy to operate, reduces sludge volume, and can eliminate pathogens (Metcalf & Eddy, 2013). For example, wastewater treatment plant digesters are mixed and heated to speed sludge mass reduction. A covered anaerobic lagoon is another option where biogas is collected using a floating cover. These lagoons are typically not heated or mixed.

Despite its benefits, controlled anaerobic digestion has not been widely adopted for reducing waste and GHGs from dairy farms due to barriers such as local regulations, fuel and electricity rates, maintenance concerns, and poor operator knowledge and skill (USDA, 2009). The USEPA estimates digesters could be installed in at least 900 dairy farms in California, but as of January 2015, only 19 anaerobic digesters were operating on California dairy farms (USEPA, 2010, 2015).

2.1.3 NO_x Emission Problems from Combusting Biogas

Another issue with controlled anaerobic digestion is that although GHGs are captured in the digesters as biogas, air pollutants, such as NO_x, carbon monoxide (CO), volatile organic compounds (VOCs), and sulfur oxides (SO_x), are emitted when biogas is combusted (Liang & Pirnie, 2009). Of most importance is NO_x, which can react with volatile organic compounds (VOCs) in the atmosphere to form ground-level ozone and smog.

NO_x emissions are a major concern in areas classified as nonattainment areas for ozone. In the San Joaquin Valley, for instance, where three-fourths of California's dairy cows are located (USEPA, 2013), the air basin is classified as an extreme nonattainment area for ozone and has the worst air quality in the nation (CalEPA, 2011; USEPA, 2013). Thus, the Air Board in this region has a strict NO_x emission limit of 9-11 ppm @ 15% O₂ for Best Available Control Technologies (BACT), or 0.15 g-NO_x/bhp-hr (CalEPA, 2011). Note: Brake horsepower (bhp) is the available power delivered by an engine at its output shaft.

Numerous NO_x control technologies are available, such as catalytic converters, selective catalytic reduction (SCR), and microturbines, but they are easily fouled by biogas contaminants (hydrogen sulfide, water vapor, other trace gases) (Jones, 2008). Therefore, the biogas needs to be refined with additional , expensive, equipment before flowing through one of these devices (ESA, 2011). Biogas refinement technologies are firmly established and regularly used for large-scale applications (such as wastewater treatment plants), but even large dairy farms could not achieve the biogas production rate these technologies are typically used for (ESA, 2011). Consequently, the implementation of anaerobic digesters on dairy farms in California has been at a near standstill in part due to the technological and cost problems of controlling air emissions (ESA, 2011).

2.1.4 Hydrogen Addition to Reduce NO_x Emissions from Farm Digesters

A potential solution to reducing NO_x emissions from IC engines is to supplement the biogas with hydrogen (H₂). Hydrogen-hydrocarbon (i.e., H₂ & CH₄) mixtures

combusted at lean air-to-fuel ratios burn with lower NO_x emissions (0.032 to 0.10 g/bhp-hr with no emission control) than CH₄ alone (0.05 to 0.15 g/bhp-hr with emission controls) (Wilson, 2012). At 15 H₂:85 CH₄ by volume, reduced NO_x emissions and engine efficiency are optimized (Choudhuri & Gollahalli, 2000; Collier et al., 1996; Shrestha & Karim, 1999; Sierens, 1998). NO_x emissions at 15:85 H₂:CH₄ are approximately 3 ppm (TerMaath, Skolnik, Schefer, & Keller, 2006), which would meet San Joaquin's current NO_x limit of 9-11 ppm and possibly more strict future limits. Hydrogen-methane mixtures burn cleaner because this allows the IC engine to burn in ultra-lean mode, resulting in lower flame temperatures that lead to less NO_x emissions than other NO_x control options (**Table 2-1**).

Table 2-1: Effect of emission controls on NO_x emissions from IC engines (Wilson, 2012).

Engine Operation	Emission Control	Impact on Emissions	NO _x (g/bhp-hr)
Stoichiometric	None	Highest NO _x emissions	8
Stoichiometric	Three-way catalyst	NO _x reacts with CO and HC on catalyst	0.15
Lean burn, prechamber	None	Lower combustion temperatures, less NO _x	0.4
Lean burn	SCR (not feasible for LFG ¹)	NO _x reacts on SCR catalyst in the presence of injected ammonia	0.05 to 0.15
Lean burn with hydrogen	None	Hydrogen in fuel extends lean operating limit	0.032 to 0.10

¹ Landfill Gas (LFG)

2.1.5 Hydrogen Production, Storage, and Transportation Issues

Some methods to produce hydrogen are steam reforming, renewable liquid reforming, electrolysis (water splitting), and dark fermentation (DOE, 2014), most of which are expensive compared with, for example, gasoline production. Steam reforming accounts for 95% of hydrogen production in the U.S, but it costs approximately three times more than gasoline per unit of energy produced. Electrolysis, another common method for producing hydrogen, is twice as expensive as steam reforming depending on electricity rates (FSEC, 2014).

Transporting hydrogen is also costly. Kornbluth et al. (2012) found the overall costs of importing commercial hydrogen for enriching landfill gas (mostly CH_4/CO_2) to reduce NO_x emissions were 45 -100% higher than NO_x control alternatives not requiring fuel purchase (e.g. lean burn, SCR, microturbine).

2.1.6 Fermentative Hydrogen Production

For hydrogen augmentation to be economically feasible, the H_2 should ideally be produced on-site. Fermentative hydrogen production, where bacteria convert an organic substrate into H_2 and CO_2 in an oxygen-free environment, such as an anaerobic digester, could potentially be a sustainable and financially viable method for producing H_2 .

The main barrier to fermentative hydrogen production is that no cost-effective method has been developed. Numerous hydrogen fermentation studies have been conducted on the laboratory scale, but many of them have suffered from low hydrogen yields (see Section 2.4.2 for a complete discussion and references).

Also, the ideal feedstocks (e.g., glucose, sucrose) are too expensive to be viable (DOE, 2004). Many studies have been conducted in batch digester mode or used pure cultures of bacteria (J. Wang & Wan, 2009), but for real-world applications, the digesters would likely be operated in continuous mode and certainly be fed non-sterile feedstocks harboring mixed bacterial cultures.

2.1.7 Two-Phase Anaerobic Digestion as the Solution

Production of hydrogen-methane mixtures on-site is possible through two-phase digestion, where two separate anaerobic digesters are operated in series. The first digester (or phase) is operated at a short hydraulic residence time (HRT) to produce H_2 , while the second-phase is a conventional, CH_4 -producing anaerobic digester. The gas from the second-phase is sparged into the first-phase to achieve an $H_2/CH_4/CO_2$ mixture. If the correct sparging rate is used, the $H_2:CH_4$ ratio in the first-phase could possibly be achieved near the ideal 15:85 $H_2:CH_4$ range for reduced NO_x emissions while maintaining acceptable engine efficiency (**Figure 2-1**).

Figure 2-1: Simplified overall proposed two-phase digestion system process using dairy manure as a potential feedstock. H₂ is generated in the first-phase by using a short HRT to washout slow-growing CH₄-producing bacteria (methanogens) while retaining H₂-producing bacteria. CH₄ is generated in the second-phase by using a long HRT to promote the growth of methanogens, which use H₂ to produce CH₄. This process is further described in Sections 2.2 and 2.3.

The main advantage of two-phase digestion is decreased reliance on expensive NOx control technologies while achieving a biogas with a very low NOx emission potential. While two-phase digestion could potentially solve the NOx problem, this system is operationally and biologically complex and requires more research to conclude if it is practical to operate.

2.2 Anaerobic Digestion Fundamentals

Anaerobic waste decomposition occurs in four general steps: (1) hydrolysis, (2) acidogenesis, (3) acetogenesis, and (4) methanogenesis (**Figure 2-2**).

Figure 2-2: The four general steps of anaerobic digestion. Overall, feedstock (organic matter) is converted to the end products CH_4 , CO_2 , and nutrient-rich digestate (remaining after anaerobic digestion). Image from (Yu & Schanbacher, 2009).

Anaerobic Digestion is an intricate process performed by a wide variety of Bacteria and Archaea (Yu & Schanbacher, 2009). Fungi and protozoa have also been discovered in anaerobic digesters, but their contribution to anaerobic digestion is unknown (Yu & Schanbacher, 2009). For simplicity, the term “bacteria” will refer to both Bacteria and Archaea.

In the first step, hydrolysis, bacteria break down the fats, proteins, and carbohydrates present in organic matter into simple, soluble organic compounds such as amino acids, monosaccharides, and fatty acids.

Next, bacteria convert the soluble organic matter into volatile fatty acids (e.g., acetic, propionic, butyric acid, also known as VFAs) alcohols, carbon dioxide,

and hydrogen gas (acidogenesis). In acetogenesis, the more complex acids and alcohols formed during the previous stage are further decomposed into acetic acid (CH_3COOH) and H_2 and CO_2 (WtERT, 2009). The most common end products (besides H_2 and CO_2) of these two stages are acetate and butyrate, the disassociated forms of acetic and butyric acid, respectively. The reactions for both products are shown below with glucose as the substrate:

Equation 2-1: Acetate Formation

Equation 2-2: Butyrate Formation

Because the first three steps of anaerobic digestion convert organic matter into H_2 , CO_2 , acetic acid, and other VFAs as end products, these processes collectively can be considered fermentative hydrogen production.

In the final step, methanogenesis, is where actual waste stabilization occurs (McCarty, 1964). Methane-forming bacteria (methanogens) convert the acetic acid and H_2 , formed in the preceding steps, into CH_4 and CO_2 . The loss of CH_4 from the waste solution is the mechanism of oxygen-demand removal (stabilization). The following equations represent the multiple pathways methanogens use to form the end-products of anaerobic digestion (WtERT, 2009):

Equation 2-3: CH_4 Formation Pathway 1

Equation 2-4: CH₄ Formation Pathway 2

Equation 2-5: CH₄ Formation Pathway 3

2.3 Two-Phase Digestion

The primary goal of this two-phase digestion study was to produce hydrogen in the first-phase digester and methane in the second-phase digester so the gases could be combined around an ideal ratio of 15 H₂: 85 CH₄. This study differed from other types of “two-stage digestion,” for example, where digestion occurs in the first digester, the second digester is used for residual sludge storage, and only CH₄ and CO₂ are the principal biogas products (Metcalf & Eddy, 2013). Other “two-phase digestion” studies separated acidogenesis from methanogenesis, but with the goal of improving system stability (Demirel & Yenigün, 2002; Ghosh, Ombregt, & Pipyn, 1985).

2.3.1 Methods to Produce Hydrogen in the First-Phase

Hydrogen is produced during the fermentation and acetogenesis stages of anaerobic digestion (fermentative hydrogen production), but its concentration is kept low by methanogens. Preventing methanogens from converting H₂ into CH₄ (2 mol H₂ used per mol CH₄ created) is vital to ensuring H₂ is released from the first-phase.

Hydrogen production in the first-phase can be encouraged through at least three methods: (1) using chemicals to inhibit methanogenic activity, (2) thermally

treating the sludge to eradicate methanogens while retaining spore-forming, H₂-producing bacteria, and (3) kinetic selection.

Chemicals and heat-treatment are impractical because they do not integrate well with two-phase digestion or are energy intensive, respectively. For example, chemicals such as chloroform, sodium 2-bromoethansulfonate (BES), and iodopropane limit methanogenic activity, but this is not desirable for two-phase digestion because the effluent from the first-phase digester will be fed into the second, CH₄-producing phase (Ruggeri, Tommasi, & Sanfilippo, 2015).

Thermally treating the inoculum is not practical because it requires a large energy input (Ruggeri et al., 2015). Also, bacteria (including methanogens) are ubiquitous and could re-enter the first-phase through the influent. Instead, a more practical and economically feasible method to creating hydrogen in the first-phase is required.

“Kinetic selection” or operating the first-phase on an HRT far shorter than that used in a CH₄-producing digester, can also result in H₂ production in the first-phase (Ruggeri et al., 2015). Ruggeri et. al (2015) found that methanogens, or H₂-consuming bacteria, have a specific growth rate ($\mu_{\max}$) of 0.055 h⁻¹, while H₂-producing bacteria have a $\mu_{\max}$ of ≈ 0.215 h⁻¹ (four times higher). This difference means methanogens need a longer HRT (usually 10 days or longer) to avoid being washed-out of a completely mixed reactor. Therefore, if the HRT is lowered to favor the growth of H₂-producing bacteria and attached growth is prevented, methanogens will be “washed out”, and H₂ will be the primary gas product of the first-phase.

Methanogenic growth is further limited at a short HRT because the accumulation of unconsumed VFAs in the digester causes the pH to become too acidic for methanogens to thrive, unless sufficient buffering capacity is provided.

Methanogens are metabolically active in a pH range of 6.6 – 7.6, but if the pH drops below 6.2, the acidity is toxic and greatly reduces CH₄ production (McCarty, 1964). Hydrogen-forming bacteria are not inhibited at acidic pHs as they operate in an optimal pH range of 5.0 – 6.5 (Valdez-Vazquez & Poggi-Varaldo, 2009).

2.4 Previous Studies on Fermentative Hydrogen Production

When operated at a short HRT, the biological process in the first-phase is essentially fermentative hydrogen production. Generating CH₄ from organic material is a well-established method, yet large-scale fermentative hydrogen production from practical organic substrates has not been accomplished (Karakashev & Angelidaki, 2011).

Numerous fermentative hydrogen production studies on glucose are available, yet there are typically major differences in inoculum, temperature, and digester type used (J. Wang & Wan, 2009), which made it difficult to decide which operational conditions are best for this project. Many experiments have used sludge as an inoculum, but the digester was operated in batch mode. Conversely, some experiments were operated in continuous mode, but these studies used pure cultures of bacteria or heat-treated sludge inoculum. Heat-treated sludge is sometimes used because it removes methanogens while retaining spore-forming, hydrogen-producing bacteria (Sung, Raskin, Duangmanee, Padmasiri, & Simmons, 2007).

This project used non-treated anaerobic digester sludge as the inoculum, was operated at 30°C (mesophilic) and used continuous-flow stirred tank reactors (CSTRs) (see Section 2.5 – Project Goals, for more details). Non-treated sludge inoculum was acceptable because the low HRT in the first-phase (theoretically) caused washout of methanogens. However, this project's conditions were unusual (especially because non-treated sludge was used), and in comparison to other studies, differences in the inoculum used, substrate, feedstock nutrient solution, or digester type existed.

Studies typically express hydrogen yield as moles of hydrogen produced per mole of glucose consumed ($\text{mol H}_2/\text{mol glucose}_{\text{consumed}}$), or molar hydrogen yield. This yield indicates how efficiently bacteria are converting glucose into H_2 . Another measure is the volumetric hydrogen yield (volume of H_2 produced per day, per volume of first-phase digester; $\text{L H}_2/\text{L-day}$).

Molar and volumetric hydrogen yields were used as benchmarks in this project. Because this project used glucose as a substrate, molar hydrogen yields allowed comparison to past research. Volumetric H_2 yields were useful for evaluation of sparging flow rates and the volumetric 15 H_2 : 85 CH_4 ratio.

Because this was a proof-of-concept project, and the ultimate goal was to prove the volumetric 15:85 H_2 : CH_4 ratio could be achieved in a two-phase system, at least with glucose, molar hydrogen yield was not as important to optimize as volumetric hydrogen yield. Molar hydrogen yield is based on glucose, but ideally

this system would be operated using wastes. Regardless, the first-phase in this project would ideally be operated at conditions optimizing both yields.

In the following sections, the ideal operational conditions and obstacles to producing H_2 from glucose are discussed further.

2.4.1 Optimal Operating Conditions

Important operating variables to consider for producing H_2 from glucose in CSTRs are the pH, HRT, and glucose loading rate. Studies generally focus on achieving the maximum molar H_2 yield on a glucose-consumed basis. The ideal values identified in previous studies for these variables are described below:

- *pH*: A lower pH than that used for methane-producing digesters is generally more beneficial to H_2 production. Fang and Liu (2002) found an optimum molar hydrogen yield of 2.1 mol H_2 /mol glucose_{consumed} was obtained at a pH of 5.5 using a mixed culture taken from an existing digester that was producing hydrogen from sucrose. Similarly, Lin and Chang (1999) found at a pH of 5.7, the highest molar yield was 1.7 mol H_2 /mol glucose_{consumed} with anaerobic sewage sludge as the inoculum. These studies used a continuous-flow digester setup with different feedstock nutrients and digester volumes than the present project used. However, even for studies that were operated in batch, used different substrates, a similar ideal pH range was identified. For example, Wang and Mu (2005) conducted batch experiments with sucrose and heat-

treated methanogenic sludge, and found the optimum pH was 5.5 with a hydrogen yield of 3.7 mol H₂/mol sucrose_{consumed}.

- *Hydraulic Residence Time:* A short HRT (less than 24 hours) is required for hydrogen fermentation because this causes slower-growing methanogens to be washed out while faster-growing, hydrogen-forming bacteria are retained. Yet, even similar CSTR experiments that used sludge inoculum and glucose as a substrate disagree on what the best HRT is. For example, Gavala et al. (2006) found an HRT of 4 hrs produced the highest molar yield of 2 mol H₂/mol glucose_{consumed}. Yet, Zhang et al. (2006) observed the highest molar hydrogen yield (1.95 mol H₂/mol glucose_{consumed}) occurred at an HRT of 10 hrs. These experiments both used 10 g glucose/L in their feedstock, but the difference in optimum HRT might be due to differences in the type and amount of feedstock nutrients used and the inoculum source.
- *Glucose Loading Rate.* Another important variable is the organic loading rate (OLR) or equivalently, when glucose is the sole organic substrate, the glucose loading rate (GLR). The units of GLR are g glucose/L-day. The equation for calculating GLR is shown below:

Equation 2-6: Glucose Loading Rate (GLR) Equation

$$\text{GLR} \left(\frac{\text{g C}_6\text{H}_{12}\text{O}_6}{\text{L digester} \cdot \text{day}} \right) = \frac{\text{Concentration of Glucose in Feedstock} \left(\frac{\text{g C}_6\text{H}_{12}\text{O}_6}{\text{L}} \right)}{\text{Hydraulic Residence Time (day)}}$$

Identifying the ideal GLR is complicated because it is dependent on two operational variables: the HRT and the glucose concentration. For testing for the best GLR, the HRT can be kept constant while changing the glucose concentration, or vice versa. Shen et al. (2009) performed a CSTR experiment using the former. The HRT was kept constant at 8 hrs while changing the glucose concentration to test loading rates between 4 – 30 g COD/L-d. This report found the highest molar hydrogen yield of 1.80 mol H_2 /mol glucose_{consumed} was obtained at 22 g COD/L-d, or roughly 20.6 g glucose/L-day (using 1.07 g COD/g glucose conversion factor). Additionally, Sreethawong et al. (2010) performed an experiment with an anaerobic sequencing batch reactor (ASBR) at a constant HRT of 24 hours and found at a GLR of 40 g/L-day, molar hydrogen yield was optimized at 1.46 mol H_2 /mol glucose_{consumed}. These two experiments used different digester types and HRTs, and they identified very different ideal GLRs. This shows that the ideal GLR is highly dependent on the HRT tested, digester type, and inoculum used, and there may not be universally best OLR for fermentative hydrogen production.

Table 2-2 summarizes the ideal operating conditions identified in all studies discussed above.

Table 2-2: Summary of ideal operating conditions for fermentative hydrogen production from the studies discussed in Section 2.4.1.

Source	Inoculum	pH	HRT (hrs)	Digester Type	GLR (g/L-day)	Molar H ₂ Yield (mol H ₂ /mol substrate _{consumed})
A	Mixed Culture	5.5	6	CSTR	28	2.1
B	Mixed Sludge	5.7	6	CSTR	75	1.7
C	Heat-Treated	5.5	N/A	Batch	N/A	3.7*
D	Heat-Treated	6	4	CSTR	60	2.0
E	Heat-Treated	5.5	10	CSTR	24	1.95
F	Mixed Sludge	5.5	8	CSTR	20.6	1.80
G	Heat-Treated	5.5	24	ASBR	40	1.46

^A Fang & Liu (2002)

^B Lin & Chang (1999)

^C Wang & Mu (2005)

^D Gavala et al. (2006)

^E Zhang et al. (2006)

^F Shen et al. (2009)

^G Sreethawong et al. (2010)

* This study used sucrose as substrate, all other studies used glucose.

2.4.2 Issue of Low Molar Hydrogen Yields

While numerous studies have produced H₂ from glucose, they have all suffered from low molar hydrogen yields. In theory, the maximum hydrogen yield that can be obtained if glucose is completely consumed is 12 mol H₂/mol glucose_{consumed}, but this reaction is not thermodynamically favorable because it has a standard gibbs free energy (ΔG°) of +241 kJ/mol glucose_{consumed} (Note: ΔG° represents conditions at 25°C, 1 atm, 1 M initial concentrations for all reactants, and pH 7.0). Instead, acetate and butyrate formation are favored, with ΔG° values of – 48 kJ/mol glucose_{consumed} and -137 kJ/mol glucose_{consumed}, respectively. While

acetate formation produces 4 mol H₂/mol glucose_{consumed} and butyrate formation produces 2 mol H₂/mol glucose_{consumed}, these amount to far less than the stoichiometric maximum of 12 mol H₂/mol glucose_{consumed}, assuming no losses in the form of new cell growth (**Table 2-3**) (Karakashev & Angelidaki, 2011).

Table 2-3: Gibbs Free Energy of Formation (ΔG°) and theoretical molar H₂ yields for the various pathways of glucose degradation in fermentative hydrogen production (Karakashev & Angelidaki, 2011).

Chemical Process for Glucose Degradation in Fermentative Hydrogen Production	Gibbs Free Energy (ΔG°)*	Theoretical H ₂ Yield (mol H ₂ /Mol glucose _{consumed})
$C_6H_{12}O_6 + 12 H_2O \rightarrow 6 HCO_3^- + 12 H_2 + 6 H^+$ (Stoichiometric)	$\frac{241 \text{ kJ}}{\text{mol}}$	12
$C_6H_{12}O_6 + 4 H_2O \rightarrow 2 CH_3COO^- + 2 HCO_3^- + 4 H_2 + 4 H^+$ (Acetate Formation)	$\frac{-48 \text{ kJ}}{\text{mol}}$	4
$C_6H_{12}O_6 + 2 H_2O \rightarrow CH_3CH_2CH_2COO^- + 2 HCO_3^- + 2 H_2 + 3 H^+$ (Butyrate Formation)	$\frac{-137 \text{ kJ}}{\text{mol}}$	2

*Note: ΔG° is for conditions of 25°C, 1 atm, 1 M initial concentrations for all reactants, and pH 7.0.

According to Mohan (2010), typical H₂ yields obtained are between 1-2 mol H₂/mol glucose, with the practical limit being 2 mol H₂/mol glucose_{consumed}. The practical limit is lower than the theoretical H₂ yields for acetate and butyrate formation. This is partly due to a portion of the energy content in glucose being converted to new cells and end-product inhibition (Mohan, 2009). As H₂ is produced in the digester, the partial pressure becomes high enough to noticeably limit H₂ production, resulting in lower molar yields (Westermann & Ahring, 2006). Also, during acidogenesis, butyrate and acetate formation occur concurrently

(see **Figure 2-2**). Since butyrate formation releases 2 mol H₂/mol glucose_{consumed} rather than the 4 mol H₂/mol glucose_{consumed} released during acetate formation, the average H₂ yield expected from fermentative hydrogen production drops closer to the practical limit of 2 mol H₂/mol glucose_{consumed}.

Theoretically, a higher yield of 4 mol H₂/mol glucose_{consumed} (also known as the “Thauer Limit”) is attainable, but this is only possible at conditions near equilibrium or extremely low partial pressures of H₂ (Hallenbeck & Benemann, 2002).

2.4.3 Methods to Increase Hydrogen Yields

Hydrogen production can be made more thermodynamically favorable by lowering the H₂ partial pressure in the fermenter. This can be accomplished by volatilizing H₂ from the growth medium by (1) increased stirring and (2) sparging with a gas other than H₂ (Das, Khanna, & Dasgupta, 2014).

Lamed et. al (1988) studied the effects of stirring on the H₂ concentration in a culture broth of *Clostridium thermocellum*. Without stirring, the H₂ concentration in the broth was three times higher than when it was stirred at 150 rpm. Also, less acetate was produced in the unstirred broth. This study suggested stirring helps transfer H₂ from the liquid phase into the gas phase (Das et al., 2014), increasing H₂ yields.

Numerous experiments have used nitrogen (N₂) and/or carbon dioxide (CO₂) to increase H₂ yields by sparging these gases into the digester liquid. Mizuno et al. (2000) conducted a nitrogen sparging experiment on a glucose-fed CSTR (2.3-L

liquid volume) and found the molar yield increased from 0.85 mol H₂/mol glucose_{consumed} without sparging to 1.43 mol H₂/mol glucose_{consumed} with sparging at 110 mL/min (2.9 L N₂/L-hr). Similarly, Kim. et al. (2006) performed a gas sparging experiment with multiple flow rates of N₂ and CO₂ (100, 200, 300, 400 mL/min) on a CSTR operated at 40 g sucrose COD/L-day and 12-hr HRT, with a liquid volume of 5.0 L. The highest H₂ yield of 1.68 mol H₂/mol hexose_{consumed} was obtained at a CO₂ sparging rate of 300 mL/min (3.6 L CO₂/L-hr) compared to 0.77 mol H₂/mol hexose_{consumed} without sparging.

2.5 Project Goals

The main goal of this project was to demonstrate that biogas with a H₂:CH₄ ratio of near 15:85 could be generated through continuous, two-phase digestion of glucose, with sparging of second-phase biogas into the first-phase reactor. The main tasks to achieve this goal were the following:

- 1. Identify the HRT and OLR in the first-phase for optimizing hydrogen yields (more details provided in Section 2.5.1)*
- 2. Test different biogas sparging rates from the second-phase into the first-phase to develop a model of hydrogen composition versus sparging rate, and use this model to predict which sparging rate achieved 15:85 H₂:CH₄*
- 3. Integrate the first and second-phases using the optimal conditions identified from the first two objectives*

The relative volume of the first and second-phase digesters was another variable needing optimization. However, due to time-constraints, a fixed first-phase: second-phase volume ratio of 1:40 was used.

2.5.1 First-Phase Optimization

Optimizing hydrogen production in the first-phase allowed for potentially more biogas to be combusted at the ideal $\text{H}_2:\text{CH}_4$ ratio than if hydrogen production was not optimized. Therefore, identifying the ideal conditions (HRT, OLR) to operate the first-phase for optimizing H_2 yields was the first objective.

2.5.2 Nitrogen Sparging

After optimizing the first-phase, the next step was to experimentally confirm that sparging increased hydrogen yields and then to identify the sparging rate of gas from the second-phase to the first-phase to obtain a $\text{H}_2:\text{CH}_4$ ratio of 15:85.

Initially, N_2 was used for sparging for convenience, and previous experiments have sparged with N_2 (Mizuno et al., 2000, Kim et al., 2006). High flow rates (far greater than could realistically be generated in the second-phase) were tested to determine the maximum achievable hydrogen yield with sparging. A more reasonable sparging rate comparable to the gas production expected from the second-phase was also tested. With these data, a model was created to identify the ideal sparging rate for obtaining 15:85 $\text{H}_2:\text{CH}_4$.

2.5.3 Integrated First and Second-Phase Operation

As described above, initially the second-phase, CH_4 -producing digesters were operated independent of the first-phase digesters to obtain baseline data and

ensure steady-state was reached. Later, when the first and second-phases were connected, the effluent from the first-phase was pumped into the second-phase, and the biogas from the second-phase was sparged into the first-phase at the target flow rate identified in the N₂ sparging experiment, with the aim of producing the desired 15:85 H₂:CH₄ ratio for reduced NO_x emissions.

CHAPTER 3: MATERIALS & METHODS

The digester system design, operations and maintenance procedures, and setups for all experiments, including a separate serum bottle experiment, are described in this chapter. Additionally, the analytical tests used for collecting experiment data, and the procedures used to create three-dimensional (3D) data models are discussed.

3.1 Apparatus & Experiment Setup

Experiments used laboratory scale anaerobic digesters fed feedstock containing glucose and other nutrients. The digester system apparatus was kept inside a laboratory hood (**Figure 3-1**) to reduce odor and evacuate biogas. Custom built gas meters measured the volume of gas exiting the digesters. Peristaltic pumps were used to pump influent and effluent through the digesters. A schematic of the first-phase system setup that will be described in Sections 3.1.1 – 3.1.5 is shown in **Figure 3-2**.

Figure 3-1: System used for conducting all first-phase and N₂ sparging experiments. The digester bottles were wrapped with heating pads covered with aluminum insulation.

Figure 3-2: Flow schematic for the first-phase digestion experiments. This particular setup was used for OLR experiments. For HRT experiments, pump tubing was configured differently (described in Section 3.1.1). Each component of the system is described in further detail in Sections 3.1.1 – 3.1.5.

3.1.1 Design of Bench-Top Anaerobic Digesters

Bench-top, continuous-flow, two-phase digesters were assembled. The first-phase digesters were 2-L fluorinated polyethylene (FLPE) bottles (Nalgene, Rochester, NY). FLPE bottles were used instead of untreated plastic bottles since fluorination prevents pressure changes in the bottle from causing the plastic walls to collapse or expand (Qorpak, 2015).

Each bottle had five holes drilled on the top shoulder for the (1) feedstock inlet, (2) effluent outlet, (3) temperature probe port, (4) sample collection port, (5) gas exit (**Figure 3-3**). An additional hole was drilled in the center of the threaded cap of the bottle for a sparging inlet. The bottle threads were wrapped with Teflon tape to prevent gas leaks. The temperature probe port hole was fitted with a 12.7-mm inner diameter (ID) barbed bulkhead fitting (Thermo Fisher Scientific, Waltham, MA) and deadend, 12.7-mm ID (3.2-mm thickness) polyvinyl chloride (PVC) tubing (Tygon, Saint-Gobain, Courbevoie, France) filled with water. The remaining holes were fitted with 6.4-mm ID barbed bulkhead fittings. All tubing was connected to the barbed bulkhead fittings and sealed with caulking (Kwik Seal, DAP) to prevent gas leaks. On the inside of the bottle, 6.4-mm ID (1.6-mm thickness) PVC (Tygon, Saint-Gobain, Courbevoie, France) tubing extended from the inlet, outlet, and sparging inlet fittings into the 1-L liquid volume of the digester. The end of the sparging inlet was fitted with a 20-mm diameter, oval air stone for N₂ or biogas sparging.

Figure 3-3: Schematic of a first-phase (H_2) anaerobic digester. The vessel rested on a stir plate and was wrapped in an electric heating pad. The protuberance shown on the top of the vessel represents the threaded cap for the bottle, which was sealed with Teflon tape to prevent gas leaks.

Seedling heat mats (Hydrofarm, Petaluma, CA) were taped onto aluminum insulation (Growers Supply, Pyersville, IA) which was wrapped around each digester with the heat mat interior to the insulation. A temperature probe compatible with the heat mat controller was submerged in the temperature port to keep the digesters at $30 \pm 2^\circ\text{C}$.

Magnetic, 3.8-cm Teflon-coated stir bars, powered by stir plates, were used to continuously mix the digesters. For first-phase optimization experiments, the stir plates (200 Mini Stirrer Cat No. 58940-158, VWR, Radnor, PA) were operated at about 1140 RPM. For the N₂ sparging and integrated experiments, different stir plates (StirStarter, Richland, Michigan) were operated at an unintentionally lower RPM of 700. However, mixing appeared vigorous in all of the first-phase digesters.

Gas generated in the digesters passed through the “gas out” fitting (**Figure 3-3**). The fitting was connected and glued with caulking to a 6.4-mm ID tube on the outside, which was linked to a 1-L Tedlar bag (Zefon International, Ocala, FL) and a tipping gas meter on the other end (**Figure 3-4**). The Tedlar bag allowed for unintended changes in the liquid volume of the digester without creating a vacuum in the headspace due to unequal feed-versus-effluent pumping and slight temperature fluctuations. A one-way valve was installed in the tubing between the Tedlar bag and gas meter to prevent water in the meter (see Section 3.1.2) from flowing into and filling up the bag and/or digester with water.

Figure 3-4: Schematic of the biogas flow. Gas produced in the digesters was directed through 6.4-mm ID tubing to a custom-built tipping gas meter. A 1-L Tedlar bag was used to accommodate changes in the liquid volume of the digester due to uneven pumping and slight temperature swings.

The second-phase (CH_4) digesters were constructed similarly to the first-phase (H_2) digesters. However, these digesters were constructed using 20-L Nalgene FLPE carboys with 20 L of liquid volume.

3.1.2 Gas Meter Design

Gas meters were used to measure the amount of gas produced by each digester. The gas meters contained two triangular chambers in a gas collection and tipping component, and the entire gas collection and tipping mechanism was submerged in a rectangular container holding water with a depth of 11.4 cm. The gas meters were designed so that each chamber filled with a specific volume needed to cause a tip, which released the biogas to the atmosphere. Each tip either opened or closed a reed switch. The switch position was recorded with a time stamp to a

data logger (Model HOBO UX120-017, Onset, Cape Cod, MA), which was periodically uploaded to a computer. The amount of biogas produced daily was calculated using the equation below:

Equation 3-1: Gas Meter Calculation

$$\text{Number of tips per day} \times \text{tipping volume} \left(\frac{\text{mL}}{\text{tip}} \right) = \text{Biogas produced per day}$$

Depending on the gas meter used, 90-110 mL of gas needed to collect in a chamber to trigger a tip. This volume was determined using a 140-mL syringe to measure the volume of air required to fill up each chamber causing it to tip. During the experiments, evaporation make-up water was added to the gas meters to keep the water level the same as during calibration. However, the gas volume measured by the data loggers could be underestimated by 100 mL (one tip) because residual gas would sometimes remain in the chamber before a tip could occur.

Figure 3-5: Schematic of gas meters used for measuring gas production from the anaerobic digesters (Fresco, 2015). Note: The dimensions used for the gas meter in this drawing are a few centimeters larger than the gas meters used for this project.

3.1.3 Leak Testing of Digesters

To minimize gas leakage during experiments, each constructed digester was tested for leaks using two methods: (1) a short-term, high-pressure test and (2) a long-term, low-pressure test.

The short-term test consisted of filling a sink with water, pressurizing a digester with N₂ to 0.4 atm (6 PSI), and submerging the digester underwater for at least a minute with all fittings closed. If no bubbles were observed escaping the digester fittings, the digester was then subjected to the long-term test.

For the long-term test, the digesters were filled with 1 L of water and all fittings except the feedstock inlet were closed. Next, a water column of approximately 61 cm (24") was connected to the inlet line. If the water level in the column did not change over the course of 12 hours or longer, the digester was considered leak-proof and ready to use.

Once the digesters were operating, a soap bubble test was used to check for leaks if a Tedlar gas bag was not fully inflating, or if a duplicate digester did not have similar gas production to its counterpart. Soapy water was sprayed onto the gas bag or digester, which was squeezed to force biogas into the gas meter. If any bubbles formed on the gas bag it was replaced with a new one. If the digester was leaking, the contents were temporarily stored in a beaker covered with Parafilm, and the leaky digester was swapped with a spare, leak-tested digester. The contents were poured into the replacement digester, and the headspace was re-purged with N₂ to reestablish an anaerobic environment.

3.1.4 Peristaltic Pump Setup

Two peristaltic pumps (Masterflex HV-07522-20, Cole-Parmer, Vernon Hills, IL) were used to pump feedstock in and effluent out of the digesters once per hour. Four pump heads (Standard Pump Head, Cole Parmer) were mounted onto the drive shaft of each peristaltic pump. Norprene tubing of 6.4-mm ID (Masterflex A-60 G L/S 24, Cole Parmer) was used with the pump heads because it was more durable than the digester tubing and could last longer before being worn out by the pump head rollers. Each pump head was connected to a digester and feedstock container (for pumping in) or sink (for pumping out).

Two pairs of digesters (four total), each tested at a different HRT or OLR, were operated at the same time (described further in Section 3.3.1) For HRT experiments (where HRT is varied), both the influent and effluent tubes for each digester operated at the same HRT were connected to the same peristaltic pump. For OLR experiments (where HRT is held constant), the influent tubes for all digesters, regardless of OLR tested, were connected to one peristaltic pump, and the effluent tubes were connected to the other pump (this is the setup shown in **Figure 3-2**).

The peristaltic pumps were calibrated by filling a feedstock reservoir with 20-L water (with tubing already connected between the container and pump head) and measuring the volume of water pumped into a graduated cylinder at a specified rotational speed and operating time. The digesters were not connected to the pump heads during calibration because it was too difficult to accurately measure

the pumped liquid volume while simulating the operating conditions of the digester, such as biogas pressure and varying liquid levels.

The peristaltic pumps were programmed to pump once per hour. The target water volume to be pumped per hourly cycle was calculated using **Equation 3-2** below:

Equation 3-2: Hourly Pumping Volume Calculation

$$\text{Volume Targeted Per Pumping Cycle (mL)} = \frac{1 \text{ L}}{\text{HRT (hr)}} \left(\frac{1000 \text{ mL}}{\text{L}} \right)$$

If the measured volume was not within $\pm 5\%$ of the targeted volume, the pump head tubing was replaced and/or a different rotational speed and pumping time were used until the $\pm 5\%$ criterion was met.

Pump head tubing wear was not excessive as the pumps did not operate continuously, but all pump head tubing was still changed out every couple of experiments as a safeguard.

3.1.5 Feedstock Reservoir Design

Sterile feedstock (recipe described in Section 3.1.7) was stored inside a refrigerator at 4°C in 20-L, FLPE reservoirs (Figure 3-6) that were continuously mixed at approximately 800 RPM with magnetic, 10.2-cm Teflon-coated stir bars powered by large magnetic stirrers (MegaMag Genie, Scientific Industries, Bohemia, NY).

Two holes (outlet ports) were drilled into the reservoir walls at about 5.1-cm from the base of the reservoir and fitted with 6.4-mm ID, barbed bulkhead fittings.

Tubing of 6.4-mm ID was connected to each bulkhead fitting. Each tube was connected to a separate pump head on the peristaltic pumps.

The reservoirs were capped, but tubing of 6.4-mm ID was connected to the reservoir cap with a plastic bulkhead fitting to allow air to flow in as the reservoir level dropped. A high-efficiency particulate air (HEPA) filter was connected to the cap tubing to prevent air particles and bacteria from contaminating the sterile feedstock.

An additional hole was drilled on the container's shoulder and fitted with a 6.4-mm bulkhead fitting. A 6.4-mm ID tube with a cap at the end was attached on the outside. In the inside of the digester, a 6.4-mm ID tube extended to approximately 7-cm from the bottom of the container. This tube was used to collect feedstock samples for testing by attaching a syringe to the end of the tube (on the outside) and drawing out liquid.

Approximately three meters of tubing connected the reservoirs to the digesters through the pump heads. The residence time of the feedstock in the tubes ranged between 36 to 144 minutes for HRTs between 6 – 36 hours. Although many procedures were performed to prevent bacterial contamination (see Section 3.3.2), visible bacterial growth would sometimes occur in the tubing lines between the feedstock reservoirs and digesters.

Figure 3-6: Schematic of a feedstock reservoir. The reservoirs were 20-L, continuously-mixed, and kept inside a refrigerator at 4°C.

3.1.6 Nitrogen Sparging Experiment Apparatus

Additional equipment was used for N₂ sparging experiments. Industrial-grade N₂ from a pressurized cylinder was used. The pressure was set to 0.4 atm (6 PSI) for all sparging rates tested. Tubing of 6.4-mm diameter connected the gas cylinder regulator to the “N₂/CH₄ In” port of the digesters.

For high sparging flow rates (> 10 L N₂/L-hr), rotameters (FM 1050 603, Matheson, Basking Ridge, NJ & Gilmont, & Gilmont Accucal GF-6541-1225, Cole-Parmer, Vernon Hills, IL) were used. These rotameters were calibrated using a primary flow meter (Drycal DC-2, Bios International, Butler, NJ) to verify which rotameter reading attained the specified sparging rate at 6 PSI.

During operation, the sparging rate calculated based on the daily volume measured by the gas meters was less than expected for high sparging flow rates. This was likely due to unnoticed leakages in the connection between the rotameter outlet fitting and tubing. Because testing high flow rates was one of the goals of this experiment, it was not essential for the expected sparging rate to match exactly with the calculated sparging rate.

At low flow rates ($< 10 \text{ L N}_2/\text{L-hr}$), a peristaltic pump (same model used for pumping liquid) was used to sparge gas into the digesters. The only low flow rate tested was 1 L/L-hr , which was too small to be measured by the primary flow meter. Therefore, the hourly volume measured by the gas meters was used to verify the sparging rate was near 1 L/L-hr .

3.1.7 Feedstock Ingredients & pH Control

The refrigerated feedstock consisted of the following ingredients:

- Anhydrous D-glucose
- Autoclaved, anaerobic digester sludge
- Sodium phosphate dibasic heptahydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$)
- Potassium phosphate monobasic (KH_2PO_4)

Anhydrous D-glucose was added to the feedstock to attain the required glucose concentration for a specific OLR or HRT tested.

In a side-experiment (Section 4.2), autoclaved, anaerobic digester sludge collected from the City of San Luis Obispo (SLO) Water Resource Recovery Facility (WRRF) (same source as inoculum, described in Section 3.1.8), was

found to be ideal for providing necessary nutrients to the bacteria, and it was added to the feedstock reservoirs at 1% (or 200 mL) of the total feedstock volume (20 L).

The chemicals $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ and KH_2PO_4 were added separately to increase the buffering capacity of the first-phase digesters so that digester pH could be kept stable. The amount of phosphate buffers to be added was estimated based on pHs measured for previous experiments and the target pH for the current experiment. In general, the combined amount of buffers added ranged between 0.05 – 0.15 M.

For some experiments, 10-N sodium hydroxide (NaOH) was added in small quantities to raise the pH of the feedstocks above the logarithmic acid dissociation constant (pK_a) of 7.2 for the phosphate buffers used. The pK_a equation for the phosphate buffering system is shown below:

Equation 3-3: pK_a equation

$$\text{pK}_a = -\log \left[\frac{[\text{H}^+][\text{HPO}_4^{-2}]}{[\text{H}_2\text{PO}_4^-]} \right]$$

At a pK_a of 7.2, the ratios of $[\text{HPO}_4^{-2}]/[\text{H}_2\text{PO}_4^-]$ are equal by definition, and $\text{pH} = \text{pK}_a$. Raising the pH of the feedstock above 7.2 increased the $[\text{HPO}_4^{-2}]$ and thus the acid buffering capacity of the feedstock because HPO_4^{-2} could accept two protons (H^+) whereas H_2PO_4^- could only accept one.

No automated devices were used to control digester pH, and the feedstock buffering system passively controlled the pH by limiting how low pH would drop.

The feedstock could not regulate the digester pH completely, so the pH would increase or decrease while staying within ± 0.20 of the average steady-state pH. At higher glucose loading rates, it was expected more acid would be generated, lowering the pH, so more buffer was added to counteract this effect (and vice versa).

3.1.8 Digester Inoculum Source

Digester inoculum sludge (bacterial seed) was taken from the unmixed, third digester in a series of three at the SLO WRRF, which treats municipal wastewater. The digested sludge was comprised of a mixture of primary clarifier sludge, trickling filter secondary clarifier sludge, and nitrifying activated sludge. All waste sludge from the clarifiers (75% of it being primary sludge) is thickened and then anaerobically digested in the three digesters with a combined solids residence time (SRT) of approximately 60 days. The sludge had an average VSS and COD of 17754 ± 2919 mg/L and 27179 ± 3539 mg/L during the one-year duration of this project, respectively.

The seed sludge was screened and used to inoculate the digesters each time a new operating variable was tested. The digesters were cleaned before being reused, so the digesters were filled with 1-L fresh sludge to begin new experiments. It was assumed the bacterial population of San Luis Obispo's (SLO) anaerobic digesters did not change significantly over the duration of an experiment and therefore did not influence results despite the inoculum being collected at different times. SLO's anaerobic digesters have a relatively long SRT (60 days) and a fairly consistent feed of municipal sludge.

3.1.9 Serum Bottle Experiment Setup

While the majority of experiments were continuous-flow, a serum bottle (batch) experiment was performed early in the project to understand why initial first-phase experiments had poor H₂ yields and acidic pH (< 5). Because these initial experiments did not use fresh inoculum to test new variables, the bacterial cultures were possibly debilitated. Some essential trace nutrient solutions may have also been missing (see Section 4.1 for description of issues). Thus, the serum bottle experiment was designed to test alternative trace nutrient solutions to increase H₂ production and gauge bacterial population health compared to fresh sludge inoculum.

The methods and apparatus described below were used for conducting the serum bottle experiment.

First, glass serum bottles (165-mL total) were filled with different types and amounts of constituents (see Section 4.2.1 for serum bottle contents) to a liquid volume of 100-mL.

To make the serum bottles anaerobic at the start of the experiment, N₂ was purged through the headspace of each bottle for one minute, and the bottles were capped with 20-mm polytetrafluoroethylene-faced butyl septa to allow for gas sampling. The serum bottles were placed in an incubator at 35 ± 2°C.

To measure gas production from the serum bottles, a 250-mL graduated cylinder was filled with water. A hole was drilled into the closed end of a 100-mL graduated cylinder and a 6.4-mm bulkhead tube fitting was attached. The 100-

mL cylinder was inverted and placed into the larger cylinder. Tubing (6.4-mm) was connected to the bulkhead fitting and extended from the closed end of the inverted cylinder to a luer-to-barb fitting and needle. The needle was inserted into the septa of the serum bottle to be measured, which caused the smaller cylinder to fill with gas and rise up in the larger cylinder. Once the cylinder stopped rising, the gas volume displaced was recorded as gas production since the last purging operation (**Figure 3-7**).

Figure 3-7: Experimental apparatus used for measuring biogas production from serum bottles (Hill, 2014). The serum bottles in this figure are from a different study using algae.

3.2 Analytical Test Methods

The following variables were measured for this project:

- *Alkalinity*: The buffering capacity of a liquid sample. Represented in units of mg CaCO_3/L
- *pH*: Measure of the $[\text{H}^+]$ concentration in a liquid sample.

- *Biogas Composition*: For measuring %H₂, %CH₄, and %CO₂ in biogas.
- *Chemical Oxygen Demand (COD)*: Indirect measurement of the amount of organic compounds in a wastewater sample.
- *Total Suspended Solids (TSS)*: Measurement of the suspended matter in water.
- *Volatile Suspended Solids (VSS)*: Measurement of the amount of suspended matter in a liquid sample that ignites at 550°C for 15 minutes.
- *Glucose Concentration*: Measurement of the soluble glucose concentration in a sample.

These analytical methods are described more fully in the following sections.

3.2.1 pH & Alkalinity

Alkalinity and pH were determined using standard methods (APHA, 2005). pH was determined using a calibrated Oakton pH probe with a gel type electrode, and alkalinity was determined per Method 2320B by titrating with 0.02 N or 0.20-N H₂SO₄ to pH 4.5 (APHA 2005).

3.2.2 Biogas Composition

Biogas composition was determined using a gas chromatograph (Model 8610, SRI, Torrance, California) equipped with a concentric column (Alltech CTR I, Deerfield, Illinois) consisting of outer and inner columns measuring 6.4-mm and 3.2-mm ID, respectively. The inner column contained an activated molecular sieve and the outer column was filled with a porous polymer mixture. Ultra-high

purity argon was used as the carrier gas. The gas chromatograph (GC) was operated at a temperature of 55°C and carrier gas flow rate of 16 mL/min. The GC was located in the same room as the digesters. Gas samples were collected by removing 1-mL of biogas from the digester headspace and injecting the sample into the GC per the bench method (Lundquist Laboratory, 2014) The sample run time was 22 minutes as methane took the longest to peak .

3.2.3 Chemical Oxygen Demand

COD was tested using the closed reflux, colorimetric method (APHA, Method 5220D, 1997). Heating blocks (Orion COD165, Thermo Fisher Scientific, Waltham, MA, & Model 163-486, Bioscience, Allentown, PA) and a colorimeter (Model DR/890, Hach, Colorado, USA) were used. All samples were digested using 0-1500 mg/L range COD reagent vials (CHEMetrics HR, Midland, Virginia). A majority of samples required dilution to be within the method range. The lower detection limit (LDL) of the COD vials was 100 mg/L with an error of $\pm 30\%$ at this limit. For COD values between 500 - 1000 mg/L, which all diluted samples were within, the error was $\pm 7.5\%$.

3.2.4 Total and Volatile Suspended Solids

TSS and VSS were measured following standard methods (APHA 2540D & 2540E, 2005) using glass fiber filters (1.2- μm pore size, G4 Grade, Fisherbrand) and a vacuum pump (Model 1HAB-25B-M100X, GAST, Benton Harbor, Michigan). Filters were weighed with a four-point balance (Model AG245, Mettler Toledo). In general, at least 10-mL of sample was needed to attain the minimum 2.5 mg dry residue on the filters per APHA.

3.2.5 Glucose Concentration

Glucose was measured using the “UV-method for Determination of D-glucose in Foodstuffs and Other Materials (R-Biopharm, 2015). A UV-VIS spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Kyoto, Japan), set to read at a wavelength of 340 nm, was used to measure the absorbance of samples.

The test’s precision was ± 0.09 g/L and ± 0.86 g/L for samples with a dilution factor (DF) of 10 and 100, respectively (R-Biopharm, 2015).

For all experiments, the measured feedstock glucose concentration was less than expected based on the amount of glucose added to the container. A possible cause was bacterial contamination in the feedstock containers, which may have caused glucose to be consumed before entering the digesters. Also, water could contaminant the anhydrous-glucose container, resulting in faulty glucose measurements. Besides contamination, it is possible the samples degraded as glucose samples were not tested until 3-12 months after being collected and stored in the freezer (the glucose bench method was developed in-house and was not finalized until near the end of the project).

To correct for this, GLR was calculated by dividing the average feedstock glucose concentration measured during the steady-state period by the HRT. The equation is expressed below:

Equation 3-4: GLR Correction Calculation

$$\text{Calculated GLR} \left(\frac{\text{g}}{\text{L-day}} \right) = \frac{\text{Avg. Glucose Concentration} \left(\frac{\text{g}}{\text{L}} \right)}{\text{HRT (hr)}/24}$$

3.2.6 Quality Control Procedures

To ensure accuracy, splits and/or matrix spikes were performed for each analytical test. Split samples passed quality control standards if the values were within 10% of each other (**Equation 3-5**).

Equation 3-5: Split Percent Error Formula

$$\% \text{ Error in Splits} = \frac{|(1\text{st Split Value}) - (2\text{nd Split Value})|}{\text{Smallest Split Value}} \times 100\%$$

For spiked samples, if the measured concentration was within 85 to 115% of the expected concentration, the sample passed (**Equation 3-6**).

Equation 3-6: Spike Percent Recovery Formula

$$\% \text{ Recovery} = \frac{\text{Spike Value Measured}}{\text{Spike Value Expected}} \times 100\%$$

Some sample sets with bad splits or spikes were passed if the discrepancies could be sufficiently attributed to poor sampling or testing technique. Sample sets that did not meet quality control criteria for unknown reasons were retested until they passed or expired.

As an additional quality control measure, blanks and standard checks were performed for all analytical tests following their respective bench methods.

3.3 Operations & Maintenance

Once the digesters were running, daily maintenance tasks were performed to ensure they were operating correctly. The procedures used for operating, maintaining, and collecting data from the digesters are described below.

3.3.1 Experiment Startup Procedure

To begin experiments, two digesters (duplicates) were filled to 1-L with anaerobic digester sludge collected from the SLO WRRF on the same day. Afterward, the digesters were sealed, placed on a stir-plate, and set to 30°C. Next, the inlet and outlet fittings were connected to pre-calibrated peristaltic pumps (calibration procedure described in Section 3.3.2). The pumps could not operate continuously at the low flow rates required. Therefore, an hourly pumping schedule was used, and the amount of liquid pumped in and out of the digesters every hour depended on the HRT.

The “gas out” line (**Figure 3-3**) was connected to a Tedlar bag and calibrated gas meter. 20-L of feedstock was prepared with anhydrous D-glucose depending on the glucose loading rate tested. The feedstock container was placed on a stir-plate inside a refrigerator at 4°C and connected to the peristaltic pump. The inlet tubing lines were primed with feedstock before turning on the pumps to ensure no air would enter the digesters.

After all connections were complete and the inlet lines were primed, pressurized N₂ (2-4 psi) was sparged through the “N₂/CH₄ In” port of the digester to make the head space anaerobic. After at least two minutes of sparging, the nitrogen

cylinder was turned off and the “N₂/CH₄ port” was quickly sealed. Finally, the influent and effluent pumps were turned on and set to pump automatically.

3.3.2 Daily Maintenance Tasks

After setting up the digesters, the pH, alkalinity, and gas production were monitored each day. Gas composition, TSS/VSS, COD, and glucose were not tested or collected until steady-state was reached (see Section 3.3.3 for explanation of steady-state).

Liquid samples for digestate and feedstock testing were collected using a syringe. Samples were placed in a beaker and promptly sealed with ParaFilm.

Gas production was measured by slowly squeezing the gas in the Tedlar bags into the gas meters. Squeezing the Tedlar bags usually took place exactly every 24 hours, but differences of 24 ± 3 hours would sometimes occur. To account for this difference, the daily gas production was normalized for the time interval.

Besides monitoring the health of the digesters, multiple maintenance tasks were performed each day to ensure the digesters were operating correctly. The most common tasks were:

1. *Reducing bacterial contamination in the feedstock and inlet lines:* The digester feed was never sterile due to the pervasiveness of bacteria. Consumption of glucose in the storage container and feed tube lines was minimized by bleaching the inlet lines periodically whenever bubbles appeared in the tubing. Additionally, the feedstock containers were

cleaned with bleach and fitted with a new HEPA air filter each time they were replaced. All measuring equipment was with desanitized with isopropyl alcohol before being used to weigh and transfer chemicals.

2. *Manually filling the digesters with feedstock or removing liquid from the digesters to ensure they were at a 1 L liquid volume:* Because the amount of liquid pumped in and out of the digester were nearly impossible to make the same due to differences in tubing age, length, or pumphead wear, a net gain or loss of ± 200 mL digester volume occurred each day.
3. *Refilling the temperature ports & gas meters with water:* The water in the temperature ports and gas meters would evaporate, so water was added to keep them filled at the correct level.
4. *Ensuring digesters & feedstock containers were stirring correctly:* Stir bars would occasionally stop spinning. This was corrected by using a magnet to realign the stir bar with the stir plate.

3.3.3 Steady State Data Collection

Alkalinity, pH and gas production were monitored daily to gauge whether the conditions inside the digester had reached steady state. Determining when steady-state was reached was important because the data collected during this period was representative of what would occur for a continuous, long-term operation of the system. The following criteria were used in determining when steady-state was reached:

- *The difference between measured pH and alkalinity values on consecutive days was less than 20%. Gas production was not considered because the gas meters only measured in 100 mL increments (per tip), and for digesters that produced little gas, the difference between one or two tips was very large.*
- *pH, alkalinity, or gas production were not following a distinct trend*
- *At least 3 HRTs had passed to ensure the first-phase digesters had selected for hydrogen-producing bacteria and washed out methanogens initially present in the inoculum*

If the steady-state criteria above were met, gas composition, and TSS/VSS were tested in addition to pH, alkalinity, and gas production for five days. COD and glucose samples were also preserved for future testing.

If one of the duplicate digesters did not meet steady-state criteria, data was still collected for both. Because each digester was subjected to the same conditions, they should have both produced similar results. In general, all measured values

for duplicate digesters were within $\pm 20\%$ of each other on a given day, with major differences being attributed to stirring problems, differences in liquid volume, or bacterial contamination in the feedstock inlet tubing.

TSS/VSS measurements of the first-phase digesters and feedstock were initially used to estimate suspended biomass in the digesters, but the VSS of the feedstock and digesters were usually too similar to each other to accurately calculate the biomass. For example, on some occasions, feedstock VSS was higher than digester VSS. Therefore, TSS/VSS data was not collected for most first-phase experiments.

3.3.4 Experiment Shutdown Procedure

Once five steady-state data points were collected, the pumps were turned off and the digesters, feedstock containers, and tubing were cleaned with bleach and rinsed with water. If a new experiment was conducted, the digesters were leak-checked before being reused.

3.3.5 Steady-State Period Verification

To confirm steady-state was reached, after an experiment the average percent hydrogen, COD, and pH were calculated for a certain time period (3-5 days at steady state). If all values for each variable in that range were within $\pm 20\%$ of the average, then these data were considered representative of the particular HRT, OLR, or sparging rate tested. Although alkalinity measurements were initially used to indicate whether the steady-state threshold was reached, alkalinity was not included in this check as there tended to be spikes or dips in alkalinity

throughout steady-state despite the other variables remaining more stable.

Glucose was not used in this verification because the feedstock containers were sometimes switched out during steady-state and bacterial contamination varied throughout an experiment.

3.3.6 Hydrogen Yield Analysis

Most hydrogen fermentation studies calculate the glucose-consumption H_2 yield ($\text{mol } H_2/\text{mol glucose}_{\text{consumed}}$) and volumetric H_2 yield ($\text{L } H_2/\text{L-day}$), but additional ways of calculating H_2 yield are useful for truly understanding which operating variable values optimized hydrogen production (**Table 3-1**).

Table 3-1: Descriptions of the types of H_2 yields analyzed for all 1st-phase optimization and N_2 sparging experiments.

Yield Name	Units	Description
Volumetric H_2 Yield	$\text{L } H_2/\text{L-day}$	Volume (L) of H_2 produced at lab-room conditions (20°C , 1 atm), normalized for digester liquid-volume (1-L)
Glucose-Introduced H_2 Yield	$\text{mol } H_2/\text{mol glucose}_{\text{introduced}}$	Amount of H_2 produced considering the amount of glucose fed, or introduced, to the bacteria
Glucose-Consumption H_2 Yield	$\text{mol } H_2/\text{mol Glucose}_{\text{consumed}}$	Amount of H_2 produced for every unit of glucose consumed. Represents glucose-utilization efficiency
COD-Introduced H_2 Yield	$\text{mol } H_2/\text{mol COD}_{\text{introduced}}$	Amount of H_2 produced considering the amount of COD fed, or introduced, to the bacteria
COD-Destroyed H_2 Yield	$\text{mol } H_2/\text{mol COD}_{\text{destroyed}}$	Amount of H_2 produced for every unit of COD destroyed. Represents substrate-utilization efficiency

The glucose-introduced H_2 yield ($\text{mol } H_2/\text{mol glucose}_{\text{introduced}}$) is useful as it shows how well the bacteria produced H_2 considering how much glucose they were fed.

Because COD was measured (and is more reliable than glucose data), the COD-introduced H_2 yield ($\text{mol } H_2/\text{mol COD}_{\text{introduced}}$) and COD-destroyed H_2 yield ($\text{mol } H_2/\text{mol COD}_{\text{destroyed}}$) are also helpful. Note: “destroyed” is used instead of “consumed” because bacteria remove, or “destroy”, soluble COD in the form of H_2 or CH_4 gas.

All of these yields are analyzed for first-phase optimization and N_2 sparging experiments.

3.4 3D Surface Modeling Procedures

3D surface models of HRT vs. OLR vs. H_2 yield were made to deduce which operating conditions maximized H_2 yield based on the experimental data collected. The source of data came from first-phase optimization experiments that were operated within a pH range of 5.0 – 6.5 (some variables were tested at $\text{pH} < 5.0$ and $\text{pH} > 6.5$).

Data collected from first-phase optimization experiments were inputted into a surface-fitting program called TableCurve3D (Systat Software, San Jose, CA). The program generated up to 1000 3D surfaces along with a corresponding equation and R^2 value. The surface chosen was the one that matched the data as closely as possible and made logical sense (e.g at an HRT or OLR of zero, hydrogen yield should be zero). H_2 yield values of zero were inputted at the

following HRT and OLRs to establish boundary limits and to create more accurate models:

- (0-hr, 0 g COD/L-day)
- (0-hr, 15 g COD/L-day)
- (0-hr, 30 g COD/L-day)
- (15-hr, 0 g COD/L-day)
- (30-hr, 0 g COD/L-day)

CHAPTER 4: FIRST-PHASE OPTIMIZATION EXPERIMENTS

First-phase optimization experiments were necessary to determine the ideal operating conditions (HRT, and OLR) for optimizing hydrogen production in the first-phase. The nitrogen sparging and integrated operation experiments could not begin without first optimizing this phase. Also, first-phase testing accounted for about 60% of the time spent on collecting data for this project. Therefore, the first-phase experiments are discussed in this chapter while the other experiments are discussed in Chapter 5.

The procedures and results for all first-phase experiments are discussed below.

4.1 Issues with Initial First-Phase Experiments

The first-phase digesters were initially operated at varying HRTs and a constant organic loading rate of 6 g glucose/L-day to determine the ideal operating conditions for producing H₂. No particular pH operating range was used (ranged between pH 4-7) because it was not clear where the ideal pH range was at the time.

Initial first-phase experiments had volumetric hydrogen yields between 0.01 – 0.47 L H₂/L-day, which were extremely low. The following major operational differences possibly contributed to this problem:

- *Open feedstock containers:* 18.9-L (five gallon) open buckets were originally used to hold the feedstock instead of 20-L, capped Nalgene containers. The buckets were mixed using a stir bar and stir plate, but the stir bars tended to stop spinning and needed to be repositioned by hand to

spin again. Readjusting the stir bar by hand could introduce bacterial contamination into the feedstock, potentially causing glucose to be consumed before it reached the digesters.

- *Low buffering capacity feedstock:* A feedstock solution containing 1.0 g/L yeast extract, 0.5 g/L peptone, 0.5 g/L meat extract, 3.2 g/L $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 1.5 g/L KH_2PO_4 , glucose, and 18-L tap water was fed to the digesters. The glucose concentration was adjusted based on the HRT tested. This feedstock was picked because the same chemicals were used for a similar two-phase digestion project using 2-L working volume digesters and a short HRT to produce H_2 (Cooney et al., 2007). The major problem with this feedstock was within a few days of preparing a new batch, the feedstock would lose all of its alkalinity and the pH would drop to less than 5 quickly. This was likely due to bacterial contamination, since bacteria produce acids as byproducts while consuming glucose.
- *Acidic digesters:* The pH in the digesters were very acidic (less than pH 5) as a result of the feedstock's poor buffering capacity and low pH.
- *Abrupt changes in HRT without reseedling:* The HRT was changed abruptly without cleaning the digesters or reseedling. The sudden change in HRT potentially "shocked" the bacteria and resulted in poor performance.

- *Steady-state collection period:* Because the HRT tended to be changed on a Friday, for convenience it was assumed the digesters were at steady-state by the following Monday, even if this was not true.
- *Digesters were not sealed or leak tested:* Caulking was not applied around the digester fittings, and digesters were never leak tested. Biogas may have leaked out of the digesters.

As a result of these issues, a serum bottle experiment was conducted to determine if better feedstock nutrients could be provided to increase H₂ yields, and to troubleshoot low H₂ yields.

4.2 Serum Bottle Experiment

The procedures, setup, and results of the serum bottle experiment, which was designed to address and resolve initial first-phase experiment issues, are discussed in this section.

4.2.1 Experimental Procedures

Eight sets of serum bottles (22 bottles total), each filled to 100-mL, were prepared for this experiment following the setup in **Table 4-1**.

The initial pH and alkalinity were tested before purging with N₂ and sealing each bottle. Afterward, biogas composition and volume were tested each day until biogas production ceased. At the end of the experiment, the serum bottles were opened and tested for pH and alkalinity.

Each serum bottle set in **Table 4-1** contained different types and amounts of inoculum (seed), substrate (feed), and nutrients. “Autoclaved Digester-3 Sludge” consisted of anaerobic digester sludge collected from the third digester at the SLO WRRF, which was autoclaved at 250°F and 20 psig for 30 minutes.

Autoclaved sludge was chosen as a possible nutrient source because it came from a healthy anaerobic digester and likely contained all the essential nutrients for bacterial growth. Autoclaving was required to ensure the sludge did not reseed the digesters or contaminate the feedstock. “Semidefined media” was the feedstock originally used for first-phase experiments. “Old seed” was made up of all the liquor from each digester used in initial first-phase experiments mixed together. Bold’s Basal Media was tested as another possible defined media. It was a medium designed for algae that was thought to be acceptable for bacteria.

Some sets contained glucose to determine hydrogen production. Sets without glucose served as controls to compare with glucose-fed sets.

Table 4-1: Setup used for the Serum Bottle Experiment to aid in determining which feedstock nutrient solution was ideal, and to troubleshoot why the initial first-phase experiments had low yields. Each serum bottle (165-mL capacity) had a total of 100-mL liquid volume.

Set #	Description of Set Tested	# of Bottles	Seed	Feed	Nutrients
0	Autoclaved Digester 3 Only	1	None	None	100-mL Autoclaved Digester 3 Sludge
1	Old Seed Only	2	100-mL Old Seed	None	None
2	Old Seed + Glucose + Semidefined Media	3	20-mL Old Seed	Media Contained Glucose (6 g/L)	80-mL Semidefined Media
3	Old Seed + Glucose + Bold's Basal Media	3	20-mL Old Seed	Media Contained Glucose (6 g/L)	80-mL Bold's Basal Media
4	Old Seed + Autoclaved Sludge	2	20-mL Old Seed	None	17-mL Autoclaved Digester 3 Sludge & 63-mL Deionized Water
5	Old Seed + Bold's Basal Media	2	20-mL Old Seed	None	80-mL Semidefined Media
6	Old Seed + Semidefined Media	3	20-mL Old Seed	None	80-mL Semidefined Media
7	Old Seed + Glucose + Autoclaved Digester 3 Sludge	3	20-mL Old Seed	75-mL of Glucose Solution (6 g/L)	5-mL Autoclaved Digester 3 Sludge
8	Digester 3 Sludge + Glucose	3	7-mL Digester 3 Sludge	93-mL of Glucose Solution (6 g/L)	Digester 3 Sludge Inoculum Provided Nutrients

4.2.2 Results

After eight days of testing gas production and composition, the serum bottles were re-opened and tested for their ending pH and alkalinity. All serum bottles experienced a decrease or stayed the same in pH, with the exception of Set 5 (Bold's Basal Media + Old Seed) which increased in pH from 5.76 to 6.34.

Alkalinity decreased for all sets that produced H₂, likely as a result of VFA production (**Table 4-2**).

Only two serum bottle sets produced significant amounts of H₂. Each bottle from Set 7 (old seed, glucose, and autoclaved D3 sludge) and Set 8 (D3 Sludge + glucose) produced an average of 266.0 and 765.3 mL H₂ per L (**Table 4-2**). By Day 5, the cumulative amount of H₂ produced in Sets 7 and 8 stayed constant until the experiment ended on Day 8 (**Figure 4-1**).

Table 4-2: pH, Alkalinity, and H₂ production results for the Serum Bottle Experiment.
This experiment lasted eight days.

#	Description of Set Tested	pH		Alkalinity (mg CaCO ₃ /L)		Total H ₂ Produced (mL H ₂)
		Start	End	Start	End	End
0	Autoclaved D3 Sludge Only	9.51	9.38	787	4700	0.0
1	Old Seed Only	6.09	6.09	1067	960	0.0
2	Old Seed + Glucose + Semidefined Media	6.81	3.58	832	0	0.4
3	Old Seed + Glucose + Bold's Basal Media	5.80	4.75	220	33	0.0
4	Old Seed + Autoclaved Sludge	8.38	7.62	920	970	0.0
5	Old Seed + Bold's Basal Media	5.76	6.34	210	467	0.0
6	Old Seed + Semidefined Media	6.81	6.60	800	1380	0.0
7	Old Seed + Gluc. + Autocl. D3 Sludge	6.97	3.19	400	0	26.6
8	Digester 3 Sludge + Glucose	8.10	4.56	655	56	76.5

Figure 4-1: Cumulative hydrogen produced per for each serum bottle set. Note: Only Set 8 and Set 7 produced significant amounts of hydrogen in this experiment.

4.2.3 Discussion

The following sets were compared to troubleshoot problems with initial first-phase experiments:

- *Set 8 vs. all other sets:* If Set 8 (only set with Digester 3 as inoculum) had a significantly higher hydrogen yield than all other bottles, it was likely the old seed was of poor quality due to abruptly changing the HRT without reseeded.
- *Set 7, Set 3, Set 2:* Comparing these sets against each other indicated if essential nutrients were missing in either of the tested feedstocks.
- *Set 2 vs. Set 6:* Set 6 and Set 2 both consisted of seed and glucose, but Set 6 excluded semi-defined media. This comparison showed if the

peptone, yeast extract, and meat extract from semi-defined media contributed to organic loading and, therefore, H₂ yields.

- *Set 0:* This set contained a single bottle filled with autoclaved digester sludge only. The purpose of this was to prove autoclaved sludge would not contaminate the feedstock or re-inoculate the digesters if it was used as a nutrient source.

The main measure of comparison for all bottles was the volumetric H₂ yield per liter of serum bottle volume. This comparison was acceptable as all bottles in each set were filled with identical ingredients and volume.

The following observations were made for this experiment:

- *The feedstock used in initial experiments lacked essential nutrients:* Set 7 (autoclaved sludge as nutrient) had significantly higher H₂ than Sets 3 and 2. Thus, it was concluded autoclaved sludge contained essential nutrients that Bold's Basal Media (Set 3) and semi-defined media (Set 2) were lacking.
- *The seed was of poor quality:* Set 8, the only bottle that did not have old bacterial seed as the inoculum, had a significantly higher H₂ yield (at least 300% higher) than all other bottles.
- *Organic carbon from peptone, yeast extract, and meat extract did not contribute significantly to yields:* No H₂ production was observed for Set 6

(semi-defined media without glucose) throughout the entire experiment.

Meanwhile, Set 2, which contained semi-defined media and glucose, produced small amounts of H_2 .

- *Autoclaved wastewater sludge did not contribute to seeding or contaminate the feedstock because it was sterile:* No biogas was made for the serum bottle containing autoclaved sludge only (Set 0). This was expected because the sludge was sterilized by autoclaving. This is important because the feedstock would not be contaminated if autoclaved sludge was used as the nutrient source. Also, the first-phase digesters would not be re-inoculated if autoclaved sludge was present in the feedstock.

4.2.4 Adjustments to Future Experiments

This experiment showed the first-phase digesters were not operated properly at the time. For future first-phase experiments, the following adjustments were made (these are detailed more fully in Chapter 3 – Materials & Methods):

- *Ensure the digesters are properly sealed to prevent any possible leaks*
- *Use autoclaved sludge as the main nutrient provider in the feedstock*
- *Continue using $Na_2HPO_4 \cdot 7H_2O$ and KH_2PO_4 as buffering agents:* Buffers are used to resist changes in pH when acid is created in the digesters, and having enough buffer was essential to ensuring the pH did not become too acidic (below pH 5).

- *Use fresh sludge from the SLO Wastewater Reclamation Facility to inoculate the digesters when changing experiment conditions:* Because numerous experiments would be conducted, it was important to use an inoculum that had a relatively constant culture of bacteria so that all experiments started on the same basis. For this reason, Digester 3 Sludge was used to inoculate future experiments.

4.3 Organic Loading Rate (OLR) Testing Experiment

This experiment was conducted to determine which glucose loading rate (GLR), and eventually, organic loading rate (OLR), maximized hydrogen yields at a constant 12-hr HRT. The results of this experiment are analyzed and discussed in the sections below.

4.3.1 Organic Loading Rate Correction

Target GLRs ranging from 4 to 30 g $C_6H_{12}O_6$ /L-day were tested at a constant HRT of 12 hours. However, measured glucose concentrations did not agree closely with the glucose expected from back-calculating with COD data (**Table 4-3**). Also, the calculated GLRs (from glucose data) typically did not correspond with the target GLRs (**Table 4-3**).

This indicated that GLR was not a reliable measure for determining which loading rate optimized hydrogen yields.

Because of these issues, the OLR (in g COD/L-day) was a better measure to use than GLR. The COD test was more established than the glucose test, COD

samples were tested a week after being collected as opposed to glucose samples being tested months after collection, and more samples were tested for COD with less deviation. Thus, the OLR (in g COD/L-day) was calculated from feedstock COD data (**Table 4-3 - rightmost column**) and was used to analyze hydrogen yields instead. Note: Because the steady-state pH range could not be completely controlled (described in Section 3.1.5), average pHs ranged from 4.39 – 6.50.

Table 4-3: Feedstock glucose concentrations, COD concentrations, and calculated GLRs for all target GLRs and pHs tested. The feedstock glucose concentration expected based on COD measurements and the organic loading rate (OLR) in terms of COD were also calculated.

Target GLR (g C ₆ H ₁₂ O ₆ /L-day)	pH	Feedstock Glucose Measured (g/L)	Calculated GLR ² (g C ₆ H ₁₂ O ₆ /L-day)	Feedstock COD Measured (mg/L)	Feedstock Glucose Calculated Based on COD ³ (g/L)	Calculated OLR ⁴ (g COD/ L-day)
4	5.77	2.34	4.7	2298.1	3.2	4.6
6	4.39	2.98	6.0	3647.8	3.6	7.3
6	6.14	2.07	4.1	3701.9	3.2	7.4
8	4.87	1.94	3.9	4066.3	1.9	8.1
12	4.24	4.78	9.6	6451.2	5.8	12.9
12	5.87	5.14	10.3	6931.0	6.2	13.9
14	5.94	5.51	11.0	7301.5	6.6	14.6
16	4.25	7.60	15.2	8344.8	8.1	16.7
16	5.83	6.65	13.3	8863.0	7.6	17.7
23	6.11	8.98	18.0	11470.3	10.5	22.9
30	6.37	12.14	24.3	15405.3	14.2	30.8

² Calculated GLR = (Feedstock Glucose Measured) / $\left(12 \text{ hr HRT} \times \frac{1 \text{ day}}{24 \text{ hr}}\right)$

³ Calculated by subtracting out 1% of the autoclaved sludge COD (0.01 x 27179.2 mg/L) from the feedstock COD (because 200-mL autoclaved sludge was present in the 20-L feedstock reservoirs, or 1%) and converting this number to g glucose/L (1.067 grams of COD per gram of glucose)

⁴ Calculated OLR = $\left(\text{Feedstock COD Measured} \times \frac{1 \text{ g COD}}{1000 \text{ mg COD}}\right) / \left(12 \text{ hr HRT} \times \frac{1 \text{ day}}{24 \text{ hr}}\right)$

4.3.2 Graphing of OLRs within pH 5.0 – 6.5

The 1st-phase digesters were unintentionally operated above and below pH 5.0 for this experiment (**Table 4-3 – 2nd column**). This occurred because the steady-state pH range could not be completely controlled (described in Section 3.1.5). Because pH 5.0 – 6.5 is typically considered optimal for hydrogen production, (J. Wang & Wan, 2009) and is more practical for real-life applications (very acidic first-phase effluent could upset the second-phase digesters), only OLRs tested within this pH range are graphed. The data obtained for OLRs at pH < 5.0 are not graphed, but are still presented and discussed.

4.3.3 Volumetric Hydrogen Yields & Hydrogen Composition

For OLRs operated at pH > 5.0, volumetric H₂ yields were zero at tested OLRs of 4.6 and 7.4 g COD/L-day. After 7.4 g COD/L-day, as OLR increased, volumetric yields increased almost linearly to a peak of 1.02 ± 0.13 L H₂/L-day at 22.9 g COD/L-day. After this point, the volumetric yield dropped to 0.77 ± 0.11 L H₂/L-day at an OLR of 30.8 g COD/L-day (**Table 4-4 & Figure 4-2**).

At low OLRs of 7.3 and 8.1 g COD/L-day operated at pH < 5.0, significantly more hydrogen was produced than similar OLRs (4.6 and 7.3 g COD/L-day) operated at pH > 5.0, which produced no biogas at all (**Table 4-4**).

The biogas H₂ composition at all OLRs except 4.6 g COD/L-day was between 40 – 70% H₂. At 4.6 and 7.4 g COD/L-day, no biogas production occurred, but 43.0% and 6.2% H₂ were still measured, respectively (**Table 4-4**).

Table 4-4: Volumetric H₂ yields, and H₂ composition measured for all organic loading rates and pHs tested at a constant 12-hr hydraulic residence time (HRT).

OLR (g COD/L-day)	pH	Percent H ₂ (%)	Vol. H ₂ Yield (L H ₂ /L-day)
4.6	5.77	6.2	0.00
7.3	4.39	55.1	0.24 ± 0.03
7.4	6.14	43.0	0.00
8.1	4.87	52.2	0.44 ± 0.11
12.9	4.24	52.9	0.32 ± 0.05
13.9	5.87	50.5	0.45 ± 0.15
14.6	5.94	57.0	0.42 ± 0.07
16.7	4.25	49.2	0.30 ± 0.07
17.7	5.83	55.4	0.76 ± 0.14
22.9	6.11	64.7	1.02 ± 0.13
30.8	6.37	67.0	0.77 ± 0.11

Figure 4-2: Volumetric H₂ yields measured for all organic loading rates (OLRs) tested at a constant hydraulic residence time (HRT) of 12 hrs. Only OLRs operated between pH 5.0 – 6.5 are graphed.

4.3.4 Glucose Consumption

Glucose consumption was calculated from influent (feedstock) glucose and effluent glucose concentrations (**Table 4-5**). Although glucose results may not have been accurate, glucose test results are still reported because digester

performance correlates with the amount of glucose introduced and utilized by bacteria. For OLRs operated at pH > 5.0, glucose consumption dropped almost linearly from 95.1% at 4.6 g COD/L-day to 45.0% at 30.8 g COD/L-day (**Figure 4-3 – dashed line**). Note: At the 4.6 and 7.4 g COD/L-day loading rates, less feedstock glucose measurements were taken because it was apparent these OLRs were not producing gas.

While percent glucose consumption decreased starting from the lowest OLR of 4.6 g COD/L-day tested, the amount of glucose consumed (in g/L) increased from 1.98 g/L at 7.4 g COD/L-day to 6.19 g/L at 22.9 g COD/L-day. At 30.8 g COD/L-day, the amount of glucose consumed decreased to 5.46 g/L (**Figure 4-3 – solid line**).

Table 4-5: Influent (feedstock) glucose, effluent glucose, glucose consumed, and % glucose consumption at all organic loading rates (OLRs) tested at a constant 12-hr hydraulic residence time (HRT).

OLR (g COD/L- day)	pH	Influent Glucose (g/L)	Effluent Glucose (g/L)	Glucose Consumed ⁶ (g/L)	% Glucose Consumed
4.6	5.77	2.34 ⁵	0.12 ± 0.18	2.23	95.1
7.3	4.39	2.98 ± 0.05	0.14 ± 0.12	2.84	95.2
7.4	6.14	2.07 ⁵	0.09 ± 0.13	1.98	95.8
8.1	4.87	1.94 ± 0.35	0.48 ± 0.38	1.46	75.5
12.9	4.24	4.78 ± 0.52	2.05 ± 0.44	2.73	57.1
13.9	5.87	5.14 ± 0.41	0.70 ± 0.73	4.43	86.3
14.6	5.94	5.51 ± 0.18	0.56 ± 0.56	4.94	89.8
16.7	4.25	7.60 ± 0.38	4.17 ± 0.45	3.43	45.1
17.7	5.83	6.65 ± 0.27	1.13 ± 0.88	5.52	83.0
22.9	6.11	8.98 ± 0.40	2.79 ± 1.04	6.19	68.9
30.8	6.37	12.14 ± 0.74	6.68 ± 1.37	5.46	45.0

⁵ Only one feedstock glucose concentration datum available as no gas was produced for this OLR

⁶ These values may not be exact due to rounding significant figures to 2 digits.

Figure 4-3: Glucose consumption for all organic loading rates (OLRs) tested between pH 5.0 – 6.5.

4.3.5 Glucose-Consumed & Glucose-Introduced Hydrogen Yields

H₂ yields on a glucose-consumed and glucose-introduced basis are discussed in this section.

On a glucose-consumed basis, at pH > 5.0, yields increased from zero to 0.56 ± 0.21 mol H₂/mol glucose_{consumed} between OLRs of 7.4 and 17.7 g COD/L-day, respectively. At OLRs higher than 17.7 g COD/L-day, yields stayed constant around 0.60 mol H₂/mol glucose_{consumed} (**Figure 4-4 – black dashed line**), indicating glucose was being converted to H₂ at the maximum possible efficiency.

On a glucose-introduced basis, at pH > 5.0, yields increased almost linearly from zero at 7.4 g COD/L-day to a peak of 0.46 ± 0.08 mol H₂/mol glucose_{introduced} at 17.7 g COD/L-day. At higher OLRs, yields decreased to 0.42 ± 0.06 mol H₂/mol glucose_{introduced} at 22.9 g COD/L-day and 0.24 ± 0.04 mol H₂/mol glucose_{introduced} at 31 g COD/L-day.

at 30.8 g COD/L-day (**Figure 4-4 – red solid line**). This decrease combined with the glucose-consumption H_2 yield staying constant between 17.7 and 30.8 g COD/L-day indicated the first-phase was overloaded at OLRs higher than 17.7 g COD/L-day.

Similar to the volumetric H_2 yield results, at OLRs of 7.3 and 8.1 g COD/L-day operated at $pH < 5.0$, yields were higher than similar OLRs of 4.6 and 7.4 g COD/L-day operated at $pH > 5.0$, which made no gas at all (**Table 4-6**).

Table 4-6: H_2 yields on a glucose-consumed and glucose-introduced basis for all organic loading rates (OLRs) and pHs tested at a constant 12-hr hydraulic residence time (HRT).

OLR (g COD/L-day)	pH	Molar H_2 Yield (mol H_2 /mol glucose)	
		Per mol glucose consumed	Per mol glucose introduced
4.6	5.77	0.00	0.00
7.3	4.39	0.29 ± 0.02	0.30 ± 0.04
7.4	6.14	0.00	0.00
8.1	4.87	2.60 ± 2.50	0.92 ± 0.39
12.9	4.24	0.42 ± 0.17	0.25 ± 0.07
13.9	5.87	0.32 ± 0.12	0.34 ± 0.14
14.6	5.94	0.32 ± 0.11	0.27 ± 0.04
16.7	4.25	0.35 ± 0.10	0.15 ± 0.03
17.7	5.83	0.56 ± 0.21	0.46 ± 0.08
22.9	6.11	0.61 ± 0.10	0.42 ± 0.06
30.8	6.37	0.62 ± 0.25	0.24 ± 0.04

Figure 4-4: H₂ yields on a glucose-consumed and glucose-introduced basis at all organic loading rates (OLRs) tested at a constant hydraulic residence time (HRT) of 12 hrs. Only OLRs operated between pH 5.0 – 6.5 are graphed.

4.3.6 COD Destruction

COD destruction was calculated from influent (feedstock) COD and effluent COD data. The % COD destroyed was less than 13% at all OLRs and pHs tested (Table 4-7).

Table 4-7: Influent (feedstock) COD, effluent COD, and % COD destruction at all organic loading rates (OLRs) tested at a constant 12-hr hydraulic residence time (HRT).

OLR (g COD/L-day)	pH	Influent COD (g/L)	Effluent COD (g/L)	% COD Destroyed
4.6	5.77	2298 ± 91	2123 ± 159	7.6
7.3	4.39	3648 ± 160	3261 ± 80	10.6
7.4	6.14	3702 ± 67	3282 ± 66	11.3
8.1	4.87	4066 ± 130	3916 ± 273	3.7
12.9	4.24	6451 ± 180	6202 ± 26	3.9
13.9	5.87	6931 ± 410	6047 ± 219	12.8
14.6	5.94	7302 ± 320	6774 ± 171	7.2
16.7	4.25	8345 ± 450	8391 ± 132	0.0
17.7	5.83	8863 ± 300	7806 ± 493	11.9
22.9	6.11	11470 ± 92	10799 ± 151	5.9
30.8	6.37	15405 ± 87.7	14965 ± 440	2.9

4.3.7 COD-Introduced & COD-Destroyed Hydrogen Yields

Hydrogen yields on a COD-destroyed ($\text{mol H}_2/\text{mol COD}_{\text{destroyed}}$) and COD-introduced ($\text{mol H}_2/\text{mol COD}_{\text{introduced}}$) basis were calculated (**Table 4-8**).

On a COD-destroyed basis, for OLRs operated at $\text{pH} > 5.0$, H_2 yield increased from zero at 4.6 g COD/L-day to $0.71 \pm 0.40 \text{ mol H}_2/\text{mol COD}_{\text{destroyed}}$ at 14.6 g COD/L-day (**Figure 4-5– black dotted line**). It is possible the first peak of $0.71 \text{ mol H}_2/\text{mol COD}_{\text{destroyed}}$ at 11.0 g COD/L-day is inflated because it had a high standard deviation of $\pm 0.40 \text{ mol H}_2/\text{mol COD}_{\text{destroyed}}$. At the next tested OLR of 17.7 g COD/L-day, the yield dipped to $0.50 \pm 0.23 \text{ mol H}_2/\text{mol COD}_{\text{destroyed}}$; however, at 22.9 g COD/L-day, the yield increased again to $1.06 \pm 0.16 \text{ mol H}_2/\text{mol COD}_{\text{destroyed}}$ at 22.9 g COD/L-day. At 30.8 g COD/L-day, the yield dropped to $0.79 \pm 0.28 \text{ mol H}_2/\text{mol COD}_{\text{destroyed}}$.

On a COD-introduced basis, for OLRs operated at $\text{pH} > 5.0$, H_2 yields rose linearly from zero at 7.4 g COD/L-day to $0.06 \pm 0.01 \text{ mol H}_2/\text{mol COD}_{\text{introduced}}$ at 17.7 g COD/L-day. The same yield of $0.06 \pm 0.01 \text{ mol H}_2/\text{mol COD}_{\text{introduced}}$ was observed at the next tested OLR of 22.9 g COD/L-day. At 30.8 g COD/L-day, the yield dropped to $0.03 \pm 0.00 \text{ mol H}_2/\text{mol COD}_{\text{introduced}}$ (**Figure 4-5 – black solid line**).

Table 4-8: H₂ yields on a COD-destroyed and COD-introduced basis for all organic loading rates (OLRs) and pHs tested at a constant 12-hr hydraulic residence time (HRT).

OLR (g COD/L-day)	pH	Molar H ₂ Yield (mol H ₂ /mol COD)	
		Per mol COD destroyed	Per mol COD introduced
4.6	5.77	0.00	0.00
7.3	4.39	0.49 ± 0.22	0.04 ± 0.01
7.4	6.14	0.00	0.00
8.1	4.87	0.51 ± 0.32	0.07 ± 0.02
12.9	4.24	0.97 ± 0.52	0.03 ± 0.01
13.9	5.87	0.24 ± 0.14	0.05 ± 0.02
14.6	5.94	0.71 ± 0.40	0.04 ± 0.01
16.7	4.25	0.80 ± 0.76	0.02 ± 0.00
17.7	5.83	0.50 ± 0.23	0.06 ± 0.01
22.9	6.11	1.06 ± 0.16	0.06 ± 0.01
30.8	6.37	0.79 ± 0.28	0.03 ± 0.00

Figure 4-5: H₂ yields on a COD-destroyed and COD-introduced basis at all organic loading rates (OLRs) tested at a constant hydraulic residence time (HRT) of 12 hrs. Only OLRs operated between pH 5.0 – 6.5 are graphed.

4.3.8 Chemical Oxygen Demand (COD) Balance

A COD balance was calculated and percent recoveries were within 88-102% for all OLRs and pHs tested (**Table 4-9 & Figure 4-6**).

Table 4-9: COD Balance for all organic loading rates (OLRs) tested. Percent (%) recovery was calculated as $\left(\frac{\text{Effluent COD} + \text{H}_2 \text{ COD Calculated}}{\text{Influent COD}} \right) \times 100\%$.

OLR (g COD/L-day)	pH	Influent COD ⁷ (g/day)	Effluent COD ⁷ (g/day)	H ₂ COD Calculated ⁸ (g/day)	% Recovery
4.6	5.77	4.6	4.2	0.00	92.4
7.3	4.39	7.3	6.5	0.16	91.6
7.4	6.14	7.4	6.6	0.01	88.9
8.1	4.87	8.1	7.8	0.29	99.9
12.9	4.24	12.9	12.4	0.21	97.8
13.9	5.87	13.9	12.1	0.30	89.4
14.6	5.94	14.6	13.5	0.28	94.7
16.7	4.25	16.7	16.8	0.20	101.7
17.7	5.83	17.7	15.6	0.51	90.9
22.9	6.11	22.9	21.6	0.68	97.1
30.8	6.37	30.8	29.9	0.51	98.8

⁷ Influent or Effluent COD (g/day) = (COD Measured ($\frac{\text{mg}}{\text{L}}$)) ($\frac{1 \text{ g COD}}{1000 \text{ mg COD}}$) ($\frac{1 \text{ L}}{\text{HRT (hr)}}$) ($\frac{24 \text{ hr}}{\text{day}}$)

⁸ H₂ COD (g/day) = (Vol. H₂ Yield ($\frac{\text{L H}_2}{\text{L-day}}$)) (1-L liquid volume) ($\frac{1}{0.08206 \text{ L-atm/mol-K}}$) ($\frac{1 \text{ atm}}{293 \text{ K}}$) ($\frac{16 \text{ g COD}}{\text{mol H}_2}$)

Figure 4-6: COD balance at all organic loading rates (OLRs) and pHs tested.

4.3.9 Discussion

Results from the OLR experiment are discussed in the paragraphs below.

Organic loading rate and pH had a noticeable effect on hydrogen yields and substrate utilization efficiency. Glucose consumption was greater than 45% at all OLRs tested, but an interesting observation is at OLRs that produced no biogas (4.6 and 7.4 g COD/L-day), all of the glucose was essentially consumed (95.1% and 95.8% consumption, respectively). These OLRs were operated at pH > 5.0, which is typically considered beneficial for H₂ production, so some biogas

production was expected. Another odd finding is at OLRs of 7.3 and 8.1 g COD/L-day, which were operated at pH < 5.0 (not typically considered an ideal pH for H₂ production), significantly more biogas was produced than for OLRs of 4.6 and 7.4 g COD/L-day, operated at pH > 5.0.

The metabolic activity of acetogens at different pHs may explain these observations. Acetogens, which consume H₂, CO₂, and/or glucose, are a type of bacteria ubiquitous in anoxic environments (Dürre, 2005), and may have been introduced into the digesters through the untreated sludge inoculum. At pH < 5.0, most acetogens are not able to grow (Dürre, 2005). Therefore, the lack of acetogenic activity at pH < 5.0 may have allowed H₂ to be produced without any bacteria consuming the gas. Conversely, at pH > 5.0, acetogens may have been actively converting H₂, CO₂ and glucose into acetate. This may explain why although glucose consumption was significant at 4.6 and 7.4 g COD/L-day (operated at pH > 5.0), no biogas was produced and the COD destruction was small.

At tested OLRs at or above 13.9 g COD/L-day, operated at pH > 5.0, hydrogen was produced even though acetogens were active in this pH range. A possible explanation why significant hydrogen production occurred above 13.9 g COD/L-day is the H₂-producing bacteria may have outperformed the acetogens at a breakpoint OLR between 7.4 and 13.9 g COD/L-day.

S.H. Kim et al. performed a similar experiment where sucrose concentration was changed from 10 – 60 g COD/L at a constant 12-hr HRT, and found acetogens

were present at 10 g COD/L with decreased H_2 production, whereas at 30 g COD/L, no acetogens were present and hydrogen production was maximized (2006). Kim et al.'s experiment and this project's results showed a breakpoint substrate concentration likely exists where acetogens are more dominant at concentrations lower than this breakpoint. For this thesis project, that breakpoint is likely at or below 13.9 g COD/L-day (or 7.0 g COD/L glucose at a 12-hr HRT), because at OLRs lower than 13.9 g COD/L-day and $pH > 5.0$, no biogas was produced.

COD destruction between the influent and effluent was relatively small (less than 13%) compared to the estimated COD destruction of 65% for a methane-producing anaerobic digester operated at a 30-day HRT (Metcalf & Eddy, 2013). However, this was expected because the glucose entering the first-phase digesters was converted to hydrogen gas, carbon dioxide, acetic acid, and other VFAs as end products (assuming acetogens were not dominant). Although some COD (16 g COD/mol H_2) exited the digesters in the form of H_2 (CO_2 has no COD), most of the COD contributed by glucose was converted to COD as VFAs, and therefore the COD destruction was relatively little. This explains why COD destruction was small compared to glucose consumption (greater than 45% at all OLRs tested).

Although COD destruction was small, attached growth, an important COD source, was not accounted for. If attached growth COD was considered, the COD destruction would likely be even smaller. Also, because Effluent COD + H_2

COD tended to be less than Influent COD, the attached growth COD could make up for this remaining difference in the COD balance.

To prove attached growth had more COD, at the end of one of the experiments a regular COD sample was collected from each duplicate first-phase digester.

Next, the digesters were shaken vigorously to dislodge attached growth into the liquid. Another COD sample was collected from each digester after shaking occurred. In comparison to the non-shaken COD samples, the shaken COD samples had an average of 14% more COD.

The glucose-consumption H_2 yields in this project were not as high as the typical values ($1-2 \text{ mol } H_2/\text{mol glucose}_{\text{consumed}}$) obtained in literature. This is likely due to the high error in glucose results, and the possibility samples degraded as they were not tested until 3-12 months after an experiment ended.

The excellent recoveries (88-102% recovery) from the COD balance showed most of the COD entering the digesters was conserved in the form of effluent COD and H_2 COD; however, the balance is slightly diminished because the influent and effluent CODs were very similar and usually within the $\pm 7.5\%$ error of the COD test. As mentioned earlier, attached growth COD was not included in the COD balance, but if it was not ignored the recoveries would be even closer to 100%. Therefore, the COD balance still likely validates data integrity.

4.3.10 Ideal Operating OLR

The goal of the OLR experiment was to determine which OLR maximized H_2 yields. At an OLR of 22.9 g COD/L-day , all H_2 yields, except on a glucose-

introduced basis, were maximized (**Table 4-10**). Therefore, 22.9 g COD/L-day was the target OLR used for all future experiments.

Table 4-10: H₂ yield summary for the ideal OLR of 22.9 g COD/L-day.

Volumetric H ₂ Yield (L H ₂ / L-day)	Molar H ₂ Yield (Glucose) (mol H ₂ /mol glucose)		Molar H ₂ Yield (COD) (mol H ₂ /mol COD)	
	Per mol glucose consumed	Per mol glucose introduced	Per mol COD destroyed	Per mol COD introduced
1.02 ± 0.13	0.61 ± 0.10	0.42 ± 0.06	1.06 ± 0.16	0.06 ± 0.01
Maximized	At Max Threshold	2nd Highest	Maximized	Maximized

4.4 Hydraulic Residence Time (HRT) Experiment

This experiment was conducted to determine which HRT maximized hydrogen yields at a constant target OLR of 22.9 g COD/L-day (determined from the previous experiment). The procedures and results of this experiment are discussed in this section.

4.4.1 Experimental Procedures

HRTs ranging from 6 – 24 hrs were tested (**Table 4-11**) while keeping OLR constant at a target of 22.9 g COD/L-day. The 18-hr HRT was repeated unintentionally at different pHs (6.33 and 6.73) to see if the same results could be reproduced. Feedstock glucose concentration was increased or decreased proportionally with HRT to keep the OLR constant. The average OLR for all HRTs tested (besides the 12-hr HRT) was 20.9 ± 1.4 g COD/L-day.

Table 4-11: Summary of hydraulic residence times (HRTs) and steady-state pHs tested for the HRT experiment. All HRTs were operated at a constant organic loading rate (OLR) of 22.9 g COD/L-day.

OLR (g COD/L-day)	HRT (hr)	pH
21.5	6	5.93
22.9	12	6.11
22.5	15	6.27
21.3	18	6.33
20.1	18	6.73
19.0	24	6.28

4.4.2 Volumetric Hydrogen Yields & Hydrogen Composition

Volumetric H₂ yield was highest (1.02 ± 0.13 L H₂/L-day) at a 12-hr HRT (same as previous experiment). At HRTs of 15 and 18 (pH 6.33) hrs, similar H₂ yields of 0.86 ± 0.11 L H₂/L-day and 0.90 ± 0.20 were calculated. At HRTs higher than 18 hours, volumetric H₂ yields decreased to roughly 0.60 L H₂/L-day. The 18-hr HRT was repeated at a higher pH of 6.73, and the yield was 0.92 ± 0.16 L H₂/L-day, which was not statistically different from 0.90 ± 0.20 L H₂/L-day obtained for the original 18-hr HRT operated at a lower pH of 6.33 (**Table 4-12 & Figure 4-7**).

Because the 18-hr HRT operated at pH 6.33 had an OLR closer to 22.9 g COD/L-day and was within the optimal pH range of 5.0 – 6.5 for H₂ production, this data set is graphed instead of the 18-hr HRT operated at pH 6.73.

Hydrogen compositions ranged between 62.9 and 80.3% H₂ for all HRTs tested (**Table 4-12**).

Table 4-12: Volumetric H₂ yields, and H₂ composition measured for all hydraulic residence times (HRTs) and pHs tested in the HRT experiment (all operated at a target organic loading rate (OLR) of 22.9 g COD/L-day).

OLR (g COD/L-day)	HRT (hr)	pH	Percent H ₂ (%)	Vol. H ₂ Yield (L H ₂ /L-day)
21.5	6	5.93	70.4	0.72 ± 0.16
22.9	12	6.11	64.7	1.02 ± 0.13
22.5	15	6.27	62.9	0.86 ± 0.11
21.3	18	6.33	67.3	0.90 ± 0.20
20.1	18	6.73	80.3	0.92 ± 0.16
19.0	24	6.28	64.7	0.56 ± 0.11

Figure 4-7: Volumetric H₂ yields measured for all hydraulic residence times (HRTs) tested at a target organic loading rate (OLR) of 22.9 g COD/L-day and between pH 5.0 – 6.5.

4.4.3 Glucose Consumption

Glucose consumption was calculated from influent (feedstock) glucose and effluent glucose concentrations (**Table 4-13**). Glucose consumption was highest at the 6-hr HRT (74.7%) and dropped linearly to 63.8% at the 15-hr HRT. After the 15-hr HRT, glucose consumption decreased to 33.7% and 24.0% at the 18 hr-HRT (pH 6.33) and 24-hr HRT (**Figure 4-8 – dashed line**).

The amount of glucose consumed increased linearly from 4.10 g/L at the 6-hr HRT to 7.68 g/L at the 15-hr HRT. At the 18-hr HRT (pH 6.33) and 24-hr HRT, the amount of glucose consumed decreased to 3.59 and 3.15 g/L, respectively (Figure 4-8 – solid line)

Table 4-13: Influent (feedstock) glucose, effluent glucose, glucose consumed, and % glucose consumption at all hydraulic residence times (HRTs) and pHs tested at a target organic loading rate (OLR) of 22.9 g COD/L-day.

HRT (hr)	pH	Influent Glucose (g/L)	Effluent Glucose (g/L)	Glucose Consumed (g/L) ¹⁰	% Glucose Consumed
6	5.93	5.48 ⁹	1.39 ± 0.12	4.10	74.7
12	6.11	8.98 ± 0.40	2.79 ± 1.04	6.19	68.9
15	6.27	12.05 ± 1.92	4.36 ± 0.54	7.68	63.8
18	6.33	10.65 ± 1.90	7.06 ± 2.44	3.59	33.7
18	6.73	14.85 ⁹	9.89 ± 0.74	4.96	33.4
24	6.28	13.13 ± 4.49	9.98 ± 1.72	3.15	24.0

⁹ Only one feedstock glucose concentration datum available

¹⁰ These values may not be exact due to rounding significant figures to 2 digits.

Figure 4-8: Glucose consumption at all hydraulic residence times (HRTs) tested between pH 5.0 and 6.5.

4.4.4 Glucose-Consumed & Glucose-Introduced Hydrogen Yields

H₂ yields on a glucose-consumed and glucose-introduced basis (**Table 4-14**) are discussed in this section.

On a glucose-consumed basis, the lowest glucose-consumed yield of 0.29 ± 0.05 mol H₂/mol glucose_{consumed} occurred at the 6-hr HRT. At higher HRTs (12, 15, 24 hrs), yields were near 0.60 mol H₂/mol glucose_{consumed}. The yield peaked at 1.22 ± 0.69 mol H₂/mol glucose_{consumed} for the 18-hr HRT operated at pH 6.33 (**Figure 4-9 – black dashed line**), but this value may have been inflated by unusually small glucose destruction in one of the digesters for one day during steady-state. Compared to the 18-hr HRT operated at pH 6.73, which had a yield of 0.84 mol H₂/mol glucose_{consumed}, the one operated at pH 6.33 had a higher yield, but 0.84 mol H₂/mol glucose_{consumed} falls within 1.22 ± 0.69 mol H₂/mol glucose_{consumed}, so the yields at both pHs may not be statistically different.

On a glucose-introduced basis, the lowest yield of 0.21 ± 0.03 mol H₂/mol glucose_{introduced} occurred at a 6-hr HRT. At a 12-hr HRT, yield rose to 0.42 ± 0.06 mol H₂/mol glucose_{introduced}, and then dropped to 0.34 ± 0.06 mol H₂/mol glucose_{introduced} at a 15-hr HRT. At a 18-hr HRT (pH 6.33), yield increased to 0.47 ± 0.14 mol H₂/mol glucose_{introduced} and dropped again to 0.28 ± 0.18 mol H₂/mol glucose_{introduced} at a 24-hr HRT (**Figure 4-9 – red solid line**). The 18-hr HRT operated at pH 6.73 had a lower yield (0.32 ± 0.10 mol H₂/mol glucose_{introduced}) than the one operated at pH 6.33 (0.47 ± 0.14 mol H₂/mol glucose_{introduced}).

Table 4-14: H₂ yields on a glucose-consumed and glucose-introduced basis for all hydraulic residence times (HRTs) and pHs tested.

HRT (hr)	pH	Molar H ₂ Yield (mol H ₂ /mol glucose)	
		Per mol glucose consumed	Per mol glucose introduced
6	5.93	0.29 ± 0.05	0.21 ± 0.03
12	6.11	0.61 ± 0.10	0.42 ± 0.06
15	6.27	0.54 ± 0.14	0.34 ± 0.06
18	6.33	1.22 ± 0.69	0.47 ± 0.14
18	6.73	0.84 ¹¹	0.32 ± 0.10
24	6.28	0.65 ± 0.22	0.28 ± 0.18

¹¹ Glucose measurements could only be attained for one digester.

Figure 4-9: H₂ yields on a glucose-consumed and glucose-introduced basis at all hydraulic residence times (HRTs) tested. Only HRTs operated between pH 5.0 – 6.5 are graphed.

4.4.5 COD Destruction

COD destruction was calculated from influent (feedstock) COD and effluent COD data. The % COD destroyed was less than 11% at all HRTs and pHs tested (Table 4-15).

Table 4-15: Influent (feedstock) COD, effluent COD, and % COD destruction at all hydraulic residence times (HRTs) tested.

HRT (hr)	pH	Influent COD (g/L)	Effluent COD (g/L)	% COD Destroyed
6	5.93	5373 ± 154	5214 ± 437	3.0
12	6.11	11470 ± 92	10799 ± 151	5.9
15	6.27	14073 ± 453	13063 ± 305	7.2
18	6.33	15961 ± 2208	14295 ± 493	10.4
18	6.73	15104 ± 142	14449 ± 224	4.3
24	6.28	18978 ± 1628	18513 ± 919	2.4

4.4.6 COD-Introduced & COD-Destroyed Hydrogen Yields

H₂ yields on a COD-destroyed and COD-introduced basis (**Table 4-16**) are discussed in this section.

On a COD-destroyed basis, H₂ yields rose from 0.43 ± 0.13 mol H₂/mol COD_{destroyed} at a 6-hr HRT to a peak of 1.06 ± 0.16 mol H₂/mol COD_{destroyed} at a 12-hr HRT. After the 12-hr HRT, H₂ yields dropped linearly to 0.32 ± 0.19 mol H₂/mol COD_{destroyed} at an 18-hr HRT (pH 6.33). At a 24-hr HRT, H₂ yield rose to 0.56 ± 0.25 mol H₂/mol COD_{destroyed} (**Figure 4-10 – black dotted line**).

Compared to the 18-hr HRT (pH 6.33), the 18-hr HRT (pH 6.73) had a significantly higher H₂ yield of 1.46 ± 0.91 mol H₂/mol COD_{destroyed}, but this value may be inflated due to unusually small COD destruction on the last-day of steady-state.

On a COD-introduced basis, H₂ yields were between 0.04 and 0.06 mol H₂/mol COD_{introduced} at all HRTs tested (**Figure 4-10 – black solid line**). The highest H₂ yields of 0.06 mol H₂/mol COD_{introduced} occurred at the 12-hr and 18-hr (pH 6.33)

HRTs. The 18-hr HRT (pH 6.73) had an identical H_2 yield of 0.06 mol H_2 /mol $COD_{introduced}$ compared to the 18-hr HRT (pH 6.33).

Table 4-16: H_2 yields on a COD-destroyed and COD-introduced basis for all hydraulic residence times (HRTs) and pHs tested.

HRT (hr)	pH	Molar H_2 Yield (mol H_2 /mol COD)	
		Per mol COD destroyed	Per mol COD introduced
6	5.93	0.43 ± 0.13	0.04 ± 0.00
12	6.11	1.06 ± 0.16	0.06 ± 0.01
15	6.27	0.68 ± 0.25	0.05 ± 0.00
18	6.33	0.32 ± 0.19	0.06 ± 0.02
18	6.73	1.46 ± 0.91	0.06 ± 0.01
24	6.28	0.56 ± 0.25	0.04 ± 0.01

Figure 4-10: H_2 yields on a COD-destroyed and COD-introduced basis at all hydraulic residence times (HRTs) tested. Only HRTs operated between pH 5.0 – 6.5 are graphed.

4.4.7 Chemical Oxygen Demand (COD) Balance

A COD balance was calculated and percent recoveries were within 92-100% (Table 4-17 and Figure 4-11) at all HRTs tested.

Table 4-17: COD Balance for all HRTs tested. Percent (%) recovery was calculated as

$$\left(\frac{\text{Effluent COD} + \text{H}_2 \text{ COD Calculated}}{\text{Influent COD}} \right) \times 100\%.$$

HRT (hr)	pH	Influent COD ¹² (g/day)	Effluent COD ¹² (g/day)	H ₂ COD Calculated ¹³ (g/day)	% Recovery
6	5.93	21.5	20.9	0.48	99.3
12	6.11	22.9	21.6	0.68	97.1
15	6.27	22.5	20.9	0.57	95.4
18	6.33	21.3	19.1	0.60	92.4
18	6.73	20.1	19.3	0.61	98.7
24	6.28	19.0	18.5	0.37	99.5

¹² Influent or Effluent COD (g/day) = (COD Measured (mg/L)) (1 g COD / 1000 mg COD) (1 L / HRT (hr)) (24 hr / day)

¹³ H₂ COD (g/day) = (Vol. H₂ Yield (L H₂ / L-day)) (1-L liquid volume) (1 / 0.08206 L-atm/mol-K) (1 atm / 293 K) (16 g COD / mol H₂)

Figure 4-11: COD balance at all hydraulic residence times (HRTs) tested.

4.4.8 Discussion

Results from the HRT experiment are discussed in the paragraphs below.

The 18-hr HRT was operated twice at pH 6.33 and pH 6.73. In both cases, the volumetric, glucose-destroyed, COD-destroyed, and COD-introduced H_2 yields were about the same. However, the glucose-introduced yield for the 18-hr HRT operated at pH 6.73 was lower than the one operated at pH 6.33. The glucose fed to 18-hr HRT (pH 6.73) was about 4 g/L higher than the 18-hr HRT (pH 6.33), so comparing glucose-introduced yields is not appropriate. Nevertheless, because the 18-hr HRT had similar H_2 yields regardless of which pH it was operated at, this suggests pH has little influence on results and it is not as important to strictly control it as long as the pH is not very acidic (pH > 6.0, maybe lower).

The steady-state pHs for all HRTs tested did not vary greatly compared to the OLR experiment, which had pHs ranging from 4.4 – 6.4. This occurred because OLR likely has more of an effect on pH than HRT. Because OLR was kept constant for the HRT experiment, as the HRT increased or decreased, the amount of glucose added increase or decreased proportionally. Thus, in any given time-period, the same amount of glucose was fed to the digesters. VFA formation, which causes pH to drop, uses glucose as a reactant, so the same amount of VFAs were probably produced in the digesters regardless of the HRT tested, causing less variation in pH.

Similar to the OLR experiment, COD destruction was less than 11% at all HRTs tested as most of the consumed glucose was converted to VFAs in the digester liquor. The actual COD destruction is likely lower because attached growth COD was unaccounted for.

4.4.9 Ideal Operating HRT

The goal of this experiment was to determine the HRT that maximized hydrogen yields in the first-phase digesters, which appeared to be 12 hrs when operated at 22.9 g COD/L-day (**Table 4-18**). At a 12-hr HRT, volumetric H₂ yield and COD H₂ yields (on a destroyed and introduced basis) were maximized. The 12-hr HRT had the third highest glucose-consumed and second-highest glucose-introduced H₂ yields, but glucose measurements were not as reliable as COD measurements.

Because the 12-hr HRT had the highest H₂ yields overall, it was chosen as the ideal operating HRT for the 1st-phase when operated at 22.9 g COD/L-day.

Table 4-18: H₂ yield summary for the ideal HRT of 12 hrs compared to other HRTs tested (within pH 5.0 – 6.5).

Volumetric H ₂ Yield (L H ₂ / L-day)	Molar H ₂ Yield (Glucose) (mol H ₂ /mol glucose)		Molar H ₂ Yield (COD) (mol H ₂ /mol COD)	
	Per mol glucose consumed	Per mol glucose introduced	Per mol COD destroyed	Per mol COD introduced
1.02 ± 0.13	0.61 ± 0.10	0.42 ± 0.06	1.06 ± 0.16	0.06 ± 0.01
Maximized	3rd Highest	2nd Highest	Maximized	At Max Threshold

4.5 3D Surface Model Summary

3D surface-models of HRT vs. OLR vs. H₂ yield within a pH range of 5.0 – 6.5 were generated using data from the OLR/HRT experiments (**Figure 4-12, Figure 4-13, Figure 4-14, Figure 4-15, Figure 4-16, all on next pages**).

The H₂ yields on the graphs do not always occur at the same operating conditions or have identical values to the ones experimentally determined. This occurred because the software extrapolates the data and does not take into account standard deviation. Also, the model must have leeway on some points so it can be practically used for estimating H₂ yields at different operating conditions (i.e. an $R^2 = 100$ polynomial with all tested points fitting perfectly could be generated, but would be useless for estimating H₂ yields at other data points). Therefore, some of the data points in the models are above or below the surface, which is indicated with a black line.

Figure 4-12: Volumetric H₂ Yield vs. HRT vs. OLR (within pH 5.0 – 6.5). This surface model predicts a maximum volumetric H₂ yield of 1.02 L H₂/L-day at a 13.0-hr HRT and an OLR of 23.2 g COD/L-day.

Figure 4-13: Glucose-consumed H₂ Yield vs. HRT vs. OLR (within pH 5.0 – 6.5). This surface model predicts a maximum glucose-consumed H₂ yield of 1.20 mol H₂/mol glucose_{consumed} at a 19.6-hr HRT and an OLR of 26.1 g COD/L-day.

Figure 4-14: Glucose-introduced H_2 Yield vs. HRT vs. OLR (within pH 5.0 – 6.5). This surface model predicts a maximum glucose-introduced H_2 yield of 0.48 mol H_2 /mol glucose_{introduced} at a 15.6-hr HRT and an OLR of 20.3 g COD/L-day.

Figure 4-15: COD-destroyed H_2 Yield vs. HRT vs. OLR (within pH 5.0 – 6.5). This surface model predicts a maximum COD-destroyed H_2 yield of 1.08 mol H_2 /mol COD_{destroyed} at an 11.6-hr HRT and an OLR of 25.2 g COD/L-day.

Figure 4-16: COD-introduced H_2 Yield vs. HRT vs. OLR (within pH 5.0 – 6.5). This surface model predicts a maximum COD-introduced H_2 yield of 0.06 mol H_2 /mol $COD_{introduced}$ at a 14.0-hr HRT and an OLR of 19.5 g COD/L-day.

The peak values for all types of H_2 yields clearly do not occur at the same OLR and HRT (**Table 4-19**), but the ideal operating conditions to maximize all yields are similar to those experimentally determined. From the previous experiments, an HRT of 12-hrs and OLR of 22.9 g COD/L-day optimized H_2 production. Based on the surface models, it was calculated an HRT of 15-hrs and OLR of 22.9 g COD/L-day was optimal (**Table 4-19 – bottom row**). These operating conditions agree closely and verify the 1st-phase experimental conclusions (that a 12-hr HRT and OLR of 22.9 g COD/L-day is optimal for H_2 production) are scientifically plausible.

Table 4-19: Maximum theoretical H₂ yield values predicted by the 3D surface models and their corresponding operating conditions.

Name of H₂ Yield	Max Yield Value Predicted By 3D Surface Model	HRT (hr)	OLR (g COD/L-day)
Volumetric	1.02 L H ₂ /L-day	13.0	23.2
Glucose-Consumed	1.20 mol H ₂ /mol glucose _{consumed}	19.6	26.1
Glucose-Introduced	0.48 mol H ₂ /mol glucose _{introduced}	15.6	20.3
COD-Destroyed	1.08 mol H ₂ /mol COD _{destroyed}	11.6	25.2
COD-Introduced	0.06 mol H ₂ /mol COD _{introduced}	14.0	19.5
	Average Value Calculated (Best Operating Conditions)	14.8	22.9

CHAPTER 5: NITROGEN SPARGING & INTEGRATED FIRST AND SECOND-PHASE EXPERIMENTS

The goal of the nitrogen sparging experiment was to determine which sparging rate achieved 15:85 H₂:CH₄ in the biogas. Once this was determined, the 1st and second-phases were operated together at this target sparging rate to prove 15:85 H₂:CH₄ could be achieved.

The operational procedures, setup, and results of these experiments are discussed in this chapter.

5.1 Nitrogen Sparging Experiment

The objectives of this experiment were to determine the ideal flow rate of gas to sparge through the first-phase digesters to achieve: (1) higher H₂ yields and (2) achieve near 15:85 H₂:CH₄. This was done by testing a wide range of sparging rates (1 – 30 L N₂/L-hr) and analyzing the H₂ yields and H₂ biogas composition. A 2D-model of H₂ composition versus sparging rate was created to estimate the sparging rate required to achieve 15:85 H₂:CH₄.

The procedures and results of this experiment are discussed below.

5.1.1 Experimental Procedures

Before conducting this experiment, a new set of 1st-phase digesters were operated at the ideal operating conditions determined in the OLR and HRT experiments (22.9 g COD/L-day, 12-hr HRT) to ensure similar yields were achieved before sparging. Once this was accomplished, the 1st-phase digesters were sparged with N₂.

Sparging rates ranging from 1 – 30 L N₂/L-hr were tested (Note: The L in the denominator represents the liquid volume of the 1st-phase, which in this case is 1-L). High sparging rates of 26.0 and 10.7 L N₂/L-hr (measured by gas meters) were tested to determine the maximum achievable H₂ yields with sparging. A low-sparging flow rate of 1.0 L N₂/L-hr was tested as this value was more likely to be attained by a second-phase digester.

The H₂ yield and biogas composition results obtained from all sparging rates were compared to those obtained for a zero-sparging rate (i.e. “no sparging”). The “no sparging” results are the same ones obtained for the 1st-phase digesters operated at 22.9 g COD/L-day and 12-hr HRT from the 1st-phase optimization experiments.

5.1.2 Organic Loading Rate and pH

OLR was calculated and pH was measured at all sparging rates (**Table 5-1**). The OLR at all sparging rates tested was between 22.7 ± 1.4 g COD/L-day. The pH at all sparging rates tested was between 6.0 – 6.4.

The target OLR was 22.9 g COD/L-day. Ideally, the pH would be 6.11 as the ideal first-phase conditions were operated at this pH, but because the pH could not be completely controlled it was acceptable for the sparged digesters to have a slightly different pH.

Table 5-1: Organic loading rates (OLRs) and pHs at all sparging rates tested. The target OLR was 22.9 g COD/L-day.

Sparging Rate (L N ₂ /L-hr)	OLR (g COD/L-day)	pH
No Sparging	22.9	6.11
1.0	22.6	6.06
10.7	24.1	6.12
26.0	21.5	6.31

5.1.3 Volumetric Hydrogen Yields

Sparging significantly increased volumetric yields at all sparging rates tested. At 1.0 L N₂/L-hr, the volumetric H₂ yield was 2.29 ± 0.43 L H₂/L-day, a 225% increase compared to the 1.02 ± 0.13 L H₂/L-day obtained without sparging for the OLR/HRT experiments. At the 10.7 and 26.0 L N₂/L-hr flow rates, volumetric yields were higher at 6.42 ± 1.37 L H₂/L-day and 4.96 ± 1.54 L H₂/L-day, respectively (**Figure 5-1**).

Figure 5-1: Volumetric H₂ yields measured at all sparging rates tested.

5.1.4 Glucose Consumption

Essentially 100% glucose consumption occurred at all sparging rates tested, compared to 68.9% glucose consumption with no sparging (**Table 5-2**).

The influent glucose values for sparged digesters varied greatly (average of 7.21 + 1.80 g/L). However, the % glucose consumption values are likely correct because effectively all of the glucose was consumed regardless of the influent glucose concentration.

Table 5-2: Influent (feedstock) glucose, effluent glucose, glucose consumed, and % glucose consumption at all sparging rates tested. Data from “no sparging” (same as 22.9 g COD/L-day, 12-hr HRT) is included for comparison.

Sparging Rate (L N ₂ /L-hr)	Influent Glucose (g/L)	Effluent Glucose (g/L)	Glucose Consumed ² (g/L)	% Glucose Consumed
No Sparging	8.98 ± 0.40	2.79 ± 1.04	6.19	68.9
1.0	8.95 ± 1.20	0.08 ± 0.06	8.87	99.2
10.7	7.53 ± 0.02	0.07 ± 0.12	7.46	99.1
26.0	5.34 ± 2.93	0.04 ± 0.02	5.30	99.3

5.1.5 Glucose-Consumed & Glucose-Introduced Hydrogen Yields

Glucose-consumption H₂ yields increased at all sparging rates tested compared to the yield obtained without sparging (0.61 ± 0.10 mol H₂/mol glucose_{consumed}). H₂ yields of 0.97 ± 0.19, 3.08 ± 0.85, and 4.26 ± 2.23 mol H₂/mol glucose_{consumed} were calculated at 1.0, 10.7, and 26.0 L N₂/L-hr, respectively (**Figure 5-2**).

Glucose-introduced H₂ yields closely matched glucose-consumption yields at all sparging rates tested as glucose consumption was essentially 100% (**Figure 5-2**).

Figure 5-2: H₂ yields on a glucose-consumed and glucose-introduced basis, and percent glucose consumed (for reference) at all sparging rates tested.

5.1.6 COD Destruction

The amount of COD destroyed was less than 19% at all sparging rates tested (Table 5-3).

Table 5-3: Influent (feedstock) COD, effluent COD, and % COD destruction at all sparging rates tested.

Sparging Rate (L N ₂ /L-hr)	Influent COD (g/L)	Effluent COD (g/L)	% COD Destroyed
No Sparging	11470 ± 92	10799 ± 151	5.9
1.0	11314 ± 358	10192 ± 262	9.9
10.7	12044 ± 1464	9798 ± 491	18.6
26.0	10732 ± 358	9482 ± 491	11.7

5.1.7 COD-Destruction & COD-Introduced Hydrogen Yields

On a COD-destroyed basis, H₂ yields increased at all sparging rates tested compared to the yield obtained without sparging (1.06 ± 0.16 mol H₂/mol COD_{destroyed}). H₂ yields of 1.53 ± 0.52 , 2.37 ± 1.26 , and 3.26 ± 2.46 mol H₂/mol

COD_{destroyed} were calculated at 1.0, 10.7, and 26.0 L N₂/L-hr, respectively (**Figure 5-3**).

On a COD-introduced basis, H₂ yields were lowest without sparging (0.06 ± 0.01 mol H₂/mol COD_{introduced}) and increased to 0.14 ± 0.03 with sparging at 1.0 L N₂/L-hr. The COD-introduced H₂ yields at higher yields of 10.7 and 26.0 L N₂/L-hr were similar at 0.35 ± 0.06 mol H₂/mol COD_{introduced} and 0.31 ± 0.10 mol H₂/mol COD_{introduced}, respectively (**Figure 5-3**).

Figure 5-3: H₂ yields on a COD-destroyed and COD-introduced basis at all sparging rates tested.

5.1.8 Chemical Oxygen Demand (COD) Balance

A COD balance was calculated and recoveries were within 96-104% for all sparging rates tested (**Table 5-4 & Figure 5-4**).

Table 5-4: COD Balance for all sparging rates tested. Percent (%) recovery was calculated as $\left(\frac{\text{Effluent COD} + \text{H}_2 \text{ COD Measured}}{\text{Influent COD}} \right) \times 100\%$.

Sparging Rate (L N ₂ /L-hr)	Influent COD ¹⁴ (g/day)	Effluent COD ¹⁴ (g/day)	H ₂ COD Calculated ¹⁵ (g/day)	% Recovery
1.0	22.63	20.38	1.53	96.8
10.6	24.09	19.59	4.28	99.1
26.0	21.46	18.96	3.30	103.7

$$^{14} \text{ Influent or Effluent COD (g/day)} = \left(\text{COD Measured (mg/L)} \right) \left(\frac{1 \text{ g COD}}{1000 \text{ mg COD}} \right) \left(\frac{1 \text{ L}}{\text{HRT (hr)}} \right) \left(\frac{24 \text{ hr}}{\text{day}} \right)$$

$$^{15} \text{ H}_2 \text{ COD (g/day)} = \left(\text{Vol. H}_2 \text{ Yield (L H}_2 \text{ /L-day)} \right) (1\text{-L liquid volume}) \left(\frac{1}{0.08206 \text{ L-atm/mol-K}} \right) \left(\frac{1 \text{ atm}}{293 \text{ K}} \right) \left(\frac{16 \text{ g COD}}{\text{mol H}_2} \right)$$

Figure 5-4: COD balance at all sparging rates tested.

5.1.9 Mixed Gas Composition

As sparging rate increased, biogas H₂ composition decreased due to dilution. The remaining constituents in the biogas were N₂ and CO₂. Because N₂ was used to simulate the second-phase biogas, it was assumed to consist of 60% CH₄ and 40% CO₂ (typical for a CH₄-producing digester). With this correction, the H₂:CH₄ ratios estimated for the 1.0, 10.6, and 26.0 L N₂/L-hr sparging rates were 15 : 83, 15 : 354, and 15 : 1083, respectively (**Table 5-5**).

Table 5-5: Measured H₂, CO₂, and N₂ biogas compositions at all sparging rates tested. Estimated mixed gas compositions and H₂:CH₄ ratios are also shown.

Sparging Rate (L N ₂ /L-hr)	Actual Phase 1 Biogas Composition			Estimated Mixed Gas Composition*			
	%H ₂	%CO ₂	%N ₂	%H ₂	%CH ₄	%CO ₂	H ₂ :CH ₄
1.0	8.6	11.8	79.6	8.6	47.8	43.6	15 :83
10.6	2.4	3.2	94.4	2.4	56.6	41.0	15 : 354
26.0	0.8	2.9	96.3	0.8	57.8	41.4	15 : 1083

*Calculated by assuming N₂ consisted of 60% CH₄, 40% CO₂.

5.1.10 Hydrogen Composition Prediction Model

Sparging rate and %H₂ were graphed to estimate the sparging rate required for a 15:85 H₂:CH₄ ratio (**Figure 5-5**). A power function with the equation $y = 9.3x^{-0.7}$, where x = sparging rate (L N₂/L-hr) and y = %H₂, was fitted to the data and had an R² of 0.99. Using this function, at a flow rate of 1.1 L gas/L-hr, a hydrogen percentage of 8.7% was expected, which corresponded to 15 H₂: 85 CH₄ if the rest of the gas is assumed to have a biogas composition comparable to the 1.0 L gas/L-hr experiment.

Figure 5-5: Actual sparging rate vs. measured %H₂ for the N₂ sparging experiment.

5.1.11 Discussion

Sparging at any of the flow rates tested increased H₂ yields as confirmed in other studies. A remarkable observance is with increasing sparging rate, the glucose-consumption yield increased drastically, with the 26.0 L N₂/L-hr flow rate achieving an H₂ yield of 4.26 ± 2.23 mol H₂/mol glucose_{consumed}. Although the standard error is high, this falls within the range of the theoretical maximum of 4 mol H₂/mol glucose. This could be due to decreased H₂ partial pressure as a result of sparging (Hallenbeck & Benemann, 2002). Decreased H₂ partial pressure makes acetate production more thermodynamically favorable, resulting in yields closer to 4 mol H₂/mol glucose (see Section 2.4.2).

Because the sparging model showed 1.1 L gas/L-hr could achieve 15:85 H₂:CH₄, this was the target sparging rate used for the integrated 1st and 2nd stage experiment. However, it should be noted that since there was only one data point

(1 L N₂/L-hr) tested near real conditions, the ideal sparging rate may not be completely accurate.

5.2 Integrated First and Second-Phase Experiment

Although two 20-L digesters were used to sparge gas into the first-phase, not enough gas was made to test the target 1.1 L gas/L-hr. Only enough gas was made to operate at a sparging rate of 0.28 L gas/L-hr, which was estimated by subtracting the gas measured in Gas Meter B (control digester) from Gas Meter A (sparged digester) (**Figure 5-6**). Therefore, 15:85 H₂:CH₄ was unlikely to be achieved. Data was still collected at this low sparging rate and is analyzed in the following sections.

5.2.1 Experimental Procedures

For the integrated experiment, two 20-L FLPE digesters, each with a liquid volume of 20 L, and HRT of 30 days, were seeded with sludge collected from the SLO WRRF. The second-phase digesters were operated for three months before connecting them to the first-phase. During this period, the second-phase digesters were manually fed effluent collected from first-phase experiments and the pH was controlled daily to keep the pH near 7.8. Alkalinity, gas production, TSS/VSS, and gas chromatography data were collected weekly.

Initially, it was planned to connect each second-phase digester to a separate first-phase digester. However, each second-phase digester on its own was not producing enough gas for sparging at the ideal rate found in the N₂ sparging experiment. Therefore, each second-phase digester was fed effluent from a

separate first-phase digester, but the gas from both second-phase digesters was combined and sparged into only one of the first-phase digesters. The non-sparged first-phase digester served as a control.

Because the first and second-phases were operated at different HRTs, a 500-mL capacity, mixed surge tank (Nalgene bottle) was placed between the first and second-phase digesters to temporarily hold effluent before it entered the second-phase. Any excess effluent collected from the first-phase overflowed out of the surge tank and was wasted because feeding all of the first-phase effluent would have made the HRT in the second-phase too short. This indicated the second-phase needed to be larger (addressed in Section 6.3.4 – Recommendations).

The biogas generated from the sparged and control first-phase digesters were collected in a gas meter and analyzed via gas chromatography. Alkalinity, pH, and COD were analyzed for all digesters once steady state was reached.

TSS/VSS was also collected from the second-phase for COD balance calculations.

Figure 5-6: Schematic of the integrated first and second-phase experiment.

5.2.2 Results

Only three-days of steady-state data were collected for this experiment due to difficulty in selecting a sparging rate (0.28 L gas/L-hr) that did not create a vacuum in the second-phase. The results (**Table 5-6**) are analyzed below.

The average pHs measured in the first-phase and second-phase were 5.71 and 6.84, respectively. The ideal pH for the first-phase was 6.11 (from OLR of 22.9 g COD/L-day at a 12-hr HRT), but the first-phase pH was lower. The sparged CO₂ from the second-phase likely entered solution and caused the pH to be lower-than-expected.

COD was measured for the first-phase and second-phase digesters. The first-phase influent (feedstock) COD was 10380 mg COD/L, which is equivalent to a 20.8 g COD/L-day OLR (target was 22.9 g COD/L-day). The effluent COD from the first-phase was 10430 mg/L, which is larger than the influent COD, but this discrepancy could be due to these values being within the COD test's $\pm 7.5\%$ accuracy error.

The difference between the influent and effluent COD in the second-phase was more pronounced than the first-phase. The influent COD was 10430 mg/L (same as first-phase effluent COD) and the effluent COD was 1220 mg/L, which means 88.3% of COD was destroyed (VFAs are converted into CH₄ in the second-phase).

Average biogas composition was measured for both phases. The biogas exiting the sparged 1st-phase digester had 25.5% H₂, 20.0% CH₄, and 54.5% CO₂,

which corresponds to an H₂:CH₄ of 15:12. The second-phase biogas consisted of 61.9% CH₄, and 38.2% CO₂.

Table 5-6: Data summary for the first and second-phases in the integrated experiment.

Phase	Influent COD (mg/L)	Eff.* COD (mg/L)	pH	%H ₂	%CH ₄	%CO ₂	Sparge Rate	H ₂ :CH ₄
1st	10380**	10430**	5.71	25.5	20.0	54.5	0.28 L gas/L-hr	15:12
2nd	10430	1220	6.84	0.0	61.9	38.2		

*"Eff." stands for "effluent."

**These CODs were within the COD test's precision error of $\pm 7.5\%$.

5.2.3 Second-Phase COD Balance

As not enough gas was made in the second-phase to sparge at 1.1 L gas/L-hr, a COD balance was calculated and theoretical gas production was estimated to determine if the second-phase digesters were functioning normally.

From the COD-balance, the COD recovery was 84.5% for the second-phase, but could have been closer to 100% because this estimation was based on the volume of CH₄ in the sparging gas, and not what was actually produced in the second-phase (**Figure 5-7**).

Figure 5-7: COD Balance for the second-phase digesters.

Theoretical gas production in the second-phase was estimated using COD data (see Appendix A for calculations). The theoretical gas production rate was 0.32 L gas/hr, compared to the sparging rate of 0.28 L gas/hr. The sparging rate was used for comparison because the gas from the second-phase was not measured directly. Thus, the actual gas produced in the second-phase was likely closer to the theoretical value as some non-sparged gas was unaccounted for.

5.2.4 Discussion

Results from the integrated experiment are discussed in the paragraphs below.

Gas production in the second-phase was too low to achieve the ideal sparging rate of 1.1 L gas/L-hr although the second-phase digesters were operating normally. The amount of gas generated in the second-phase could be increased by adding more substrate, but due to time constraints this solution was not performed. Thus, without any operational or reactor changes, an additional 120 L (160 L total) of suspended mix, second-phase digesters would be required to sparge enough gas to achieve 15:85 H₂:CH₄ in one 1-L first-phase digester.

COD destruction in the second-phase was significantly higher (88%) than the first-phase (not measurable). Because most of the COD in anaerobic digestion is converted into CH₄, this explains why the COD destruction was high.

The sparging rate for this experiment was 0.28 L gas/L-hr and the %H₂ measured was 25.5%, which was plugged into the nitrogen sparging model. At 0.28 L gas/L-hr, 22.3% H₂ was predicted by the nitrogen sparging model. The percent difference between the measured and predicted %H₂ at this sparging rate was 14.3%, indicating the model was relatively accurate for predicting H₂ composition at different sparging rates (**Figure 5-8**). Therefore, although the ideal sparging rate of 1.1 L/L-hr was not tested, it is still likely a sparging rate near this value can achieve the ideal 15:85 H₂:CH₄ ratio for the specific conditions used in this project.

Figure 5-8: Measured and predicted %H₂ at a sparging rate of 0.28 L gas/L-hr.

CHAPTER 6: CONCLUSIONS

In this chapter, the optimal operating conditions from (1) first-phase optimization experiments, (2) N₂ sparging experiments, and (3) integrated operation of the 1st and second-phase to obtain a 15:85 H₂:CH₄ ratio are summarized. Research limitations and recommendations for future studies are also discussed.

6.1 Experimental Conclusions

The following experimental conclusions were made for each of this project's goals (*italicized*):

- *Identify the HRT and OLR in the first-phase for optimizing hydrogen yields:*
An OLR of 22.9 g COD/L-day, 12-hr HRT, and pH of 6.11 optimized hydrogen yields in the first-phase (**Table 4-18**).
- *Test different biogas sparging rates from the second-phase into the first-phase to develop a model of hydrogen composition versus sparging rate, and use this model to predict which sparging rate achieved 15:85 H₂:CH₄:*
A sparging rate of 1.1 L gas/L-hr from the second-phase to the first-phase was estimated to obtain the ideal 15 H₂:85 CH₄ ratio.
- *Integrate the first and second-phases using the optimal conditions identified from the first two objectives:* A sparging rate of 0.28 L gas/L-hr could only be achieved for the integrated experiment. At this sparging rate, the H₂:CH₄ ratio was 15:12, yet the results agreed closely with the model used for predicting the optimal sparging rate was 1.1 L gas/L-hr. Therefore, a sparging rate near 1.1 L gas/L-hr could still potentially achieve 15:85

H₂:CH₄ in the biogas for the specific operating conditions used in this project.

6.2 Research Limitations

A few limitations encountered and identified in this study were:

- The HRT of the first-phase (12 hrs) was significantly faster than the 2nd phase (30 days), so a surge tank was required to temporarily store first-phase effluent. Excess first-phase effluent had to be wasted as the second-phase digesters could not accommodate all of it.
- Not enough biogas was produced in the second-phase to test the ideal sparging rate of 1.1 L gas/L-hr although it was operating normally. More biogas could have been produced by manually adding glucose into the second-phase, but this was not done due to time constraints.
- Attached growth (biofilm) was observed in all first-phase digesters. This presents a problem because methanogens can become attached and eventually dominate the first-phase. Although no methane was measured in the first-phase experiments, the operation time of the digesters was never longer than three weeks. Long term-operation of the digesters could potentially result in takeover of the first-phase by methanogens
- This was a proof-of-concept project, and thus, the ideal operating conditions identified in this study only apply to the specific experiment setup used.

- The mixing speed was not the same for all experiments due to unintentionally using different types of stir plates. For first-phase optimization experiments, the stir plates had an RPM of around 1140. For the N₂ sparging and integrated experiments, the first-phase digesters were operated with stir plates at an RPM of approximately 700.
- The mixing speeds tested in this experiment do not accurately reflect realistic operation of the first-phase. Mixing speeds would be lower due to the higher costs and technological limitations of mixing fast at full-scale.

6.3 Recommendations

Recommendations for future studies on (1) two-phase digestion and (2) hydrogen-methane mixtures for reducing NO_x emissions in biogas will be discussed below.

6.3.1 Attached Growth Research

While it was assumed only suspended biomass occurred in the digesters, growth was observed on the walls and tubing, most likely originating as residue from the sludge inoculum (**Figure 6-1**). Therefore, methanogens may have been present in the first-phase because the SRT of the digesters was likely longer than the HRT due to biofilm growth.

Figure 6-1: Attached growth occurred on the tubing in the inside of the digesters and the inner walls.

No methane was measured for all first-phase experiments that produced measurable amounts of gas. This could potentially be due to individual OLR/HRT experiments typically lasting no longer than three weeks. The low pHs (less than 6.5 in general) tested in the first-phase may have also suppressed methane production as methanogens prefer a pH range of 6.6 – 7.6.

Some hydrogen fermentation studies observed no methane production even when operated for months, but these studies used heated and chemically-treated inoculum to eliminate methanogens (Chang, Lee, & Lin, 2002; C. Y. Lin, Lee, Tseng, & Shiao, 2006). Conversely, Fang and Liu used a non-treated, mixed culture inoculum and found that even at a 6-hr HRT, methane production occurred at pHs above 6.0 (2002).

For future studies on two-phase digestion, it needs to be confirmed if methanogens are suppressed in the first-phase even with attached growth for long-term operation.

6.3.2 Practical Substrates for Hydrogen Production

Glucose was used as the substrate in this experiment because it was easy to measure, readily consumed by bacteria, and numerous studies on producing hydrogen from glucose are available. However, for real-life applications, glucose is not practical as it needs to be purchased and the cost-benefit ratio would not justify anaerobically digesting it. More suitable substrates for producing hydrogen, preferably organic wastes such as food waste, corn stock waste or human waste, need to be studied for use in two-phase digestion.

Manure was not mentioned because a separate in-house study found manure is not able to produce hydrogen. As anaerobic digestion occurs in a cow's digestive system, the products left over (mainly VFAs) are not suitable for hydrogen production. Methane can still be produced from manure, but a different substrate needs to be digested to produce hydrogen in the first-phase (see Section 6.3.3).

6.3.3 Digestion of Different Substrates in the First-Phase

Because digested manure is capable of producing methane but not hydrogen, for application of two-phase digestion at a dairy farm, other cheap, practical substrates would need to be explored for use in the first-phase. Dairy farms that transport milk to creameries could potentially haul back creamery waste for use in the first-phase. Another potential substrate is glycerol, a waste product of biodiesel production (Donkin, Koser, White, Doane, & Cecava, 2009). Glycerol is widely available and its price has recently dropped (Adhikari, Fernando, & Haryanto, 2009), making it a substrate worth investing more research into.

6.3.4 Fixed Film Second-Phase Digesters

Because the first-phase HRT (12 hrs) was much faster than the second-phase (30 days), the second-phase could not accommodate all of the first-phase effluent. As a result, first-phase effluent was discharged from the system via surge tanks.

For practical applications, all of the waste would need to be accommodated by the second-phase. Using fixed-film digesters instead of suspended growth digesters could potentially solve this problem. Fixed-film digesters are filled with media to promote attached growth, which effectively decouples the SRT from the HRT. Because fixed-film digesters retain more bacteria than suspended-growth digesters by volume, waste is degraded faster. This allows for fixed-film digesters to be operated at typical HRTs of 2-6 days and less volume than suspended-growth digesters (Wilkie, 2000).

6.3.5 Digester Volume Ratio Optimization

A major problem encountered in this project was not enough gas was produced in the second-phase digesters to sparge at the ideal rate of 1.1 L gas/L-hr.

Although the first-phase to second-phase liquid to volume ratio was 1:40, this ratio would need to be at least 1:160 to attain the ideal sparging rate if no operational changes are made (e.g. operating the 1st-phase so less hydrogen is made and therefore less sparging gas is required to achieve 15:85 H₂:CH₄). As mentioned in Section 6.3.4, fixed-film second-phase digesters could potentially reduce the required volume so the first-to-second-phase volume ratio is substantially lower.

Another option is to utilize an existing covered anaerobic lagoon as a second-phase digester, as the first-phase digester's volume could realistically be 1/160th of the lagoon's volume.

6.3.6 Methane Reformation of Biogas to Hydrogen

Biogas reformation could potentially be a simpler, more cost-effective approach to producing hydrogen-methane mixtures than two-phase digestion. Methane reformation is currently the most used commercial method for producing hydrogen (Holladay, Hu, King, & Wang, 2009). However, raw biogas cannot be used in traditional reforming processes as catalysts can be poisoned by hydrogen sulfide present in the gas (Alves et al., 2013). Also, biogas with a CH_4/CO_2 ratio > 1 causes coke formation and plugging of nickel-based catalysts (Effendi, Hellgardt, Zhang, & Yoshida, 2005).

In all cases, H_2S needs to be removed from biogas to be used in any reforming process. This is similar to how biogas needs to be purified for acceptable use in an internal combustion engine. Additional research is needed to determine whether it is more financially feasible and easier to reform biogas from a conventional anaerobic digester into hydrogen than using two-phase digestion to produce hydrogen-methane mixtures.

6.3.7 Effect of CO_2 on Combustion Characteristics of H_2/CH_4 Blends

The ideal 15:85 $\text{H}_2:\text{CH}_4$ ratio for reduced NO_x emissions and optimal engine efficiency was determined from studies that used only hydrogen and methane.

However, because CO_2 is also present in the biogas, it could negatively affect the combustion characteristics of the gas.

One negative aspect of CO_2 is that it cannot be burned with oxygen, lowering the heating value of the gas mixture. Also, biogas containing more than 40% CO_2 needs to be scrubbed to prevent poor engine performance (Bari, 1996), leading to increased costs. Yet, the presence of CO_2 is not completely bad because less NO_x emissions (but more hydrocarbons) are generated from combusting biogas containing CO_2 than natural gas (mainly CH_4) alone (Crookes, 2006).

Regardless, for biogas mixtures containing H_2 - CH_4 mixtures for reduced NO_x emissions, further research is needed to determine the effect CO_2 has on combustion characteristics and pollutant emissions if it is present in the gas during combustion.

6.3.8 Effect of Mixing on H_2 Production

For this study, high mixing speeds (700 and 1140 RPM) were used unintentionally. High mixing speeds lower the liquid-side hydrogen partial pressure (Cooney et al., 2007), increasing hydrogen production. For full-scale hydrogen-producing digesters, lower mixing speeds are expected (Cooney et al., 2007). Therefore, additional research on two-phase digestion using slower, more realistic mixing speeds is needed to better understand how this process would scale-up.

6.3.9 Lactic Acid Bacteria

A similar two-phase digestion study that used non-treated inoculum had considerable amounts of lactate present, likely due to the presence of lactic acid bacteria, which consume substrate but do not produce hydrogen. As a result, this study had low H₂ yields (Cooney et al., 2007). Because the feedstock and inlet lines had to be replaced every two days due to visible bacterial contamination, and the mixing speeds were intentionally low (increasing the H₂ partial pressure), lactic-acid bacteria were favored (Cooney et al., 2007).

For this thesis, visible contamination never occurred in the improved feedstock containers, and inlet tubing lines were cleaned occasionally (every 3-4 days) to discourage bacterial growth. Therefore, lactic acid bacteria may not have been as dominant in this project as the one performed by Cooney et al.

While lactic-acid bacteria did not seem to affect hydrogen production in this thesis, for future studies, it is important to ensure their growth is limited to prevent low H₂ yields.

6.3.10 Volatile Fatty Acid Testing

VFAs were not tested for this project, but for future studies it is recommended to test for these components as the relative amounts of acetic and butyric acid directly relate to bacterial metabolism and hydrogen production (see **Table 2-2** for conversion of glucose to hydrogen equations).

6.3.11 Testing Realistic Sparging Rates

Sparging rates between 1 – 30 L N₂/L-hr were tested for the nitrogen sparging rate experiment, but in reality, rates between 0.5 and 2.0 L gas/L-hr would be more likely obtained for a normal digestion system. Thus, it is recommended that for future research on generating H₂-CH₄ mixtures with two-phase digestion, a sparging experiment using the rates above be performed to verify if the ideal sparging rate is actually 1.1 L gas/L-hr.

REFERENCES

- Adhikari, S., Fernando, S. D., & Haryanto, A. (2009). Hydrogen production from glycerol: An update. *Energy Conversion and Management*, 50(10), 2600–2604. <http://doi.org/10.1016/j.enconman.2009.06.011>
- Alves, H. J., Bley Junior, C., Niklevicz, R. R., Frigo, E. P., Frigo, M. S., & Coimbra-Araújo, C. H. (2013). Overview of hydrogen production technologies from biogas and the applications in fuel cells. *International Journal of Hydrogen Energy*, 38(13), 5215–5225. <http://doi.org/10.1016/j.ijhydene.2013.02.057>
- APHA. (2005). *Standard Methods for the Examination of Water and Wastewater*, 21st ed. Washington, D.C.: American Public Health Association.
- Bari, S. (1996). Effect of carbon dioxide on the performance of biogas/diesel duel-fuel engine. *Renewable Energy*, 9(1-4), 1007–1010. [http://doi.org/10.1016/0960-1481\(96\)88450-3](http://doi.org/10.1016/0960-1481(96)88450-3)
- Bracmort, K. (2010). Anaerobic Digestion : Greenhouse Gas Emission Reduction and Energy Generation. Retrieved from <http://nationalaglawcenter.org/wp-content/uploads/assets/crs/R40667.pdf>
- CalEPA. (2011). *Permit Guidance For Anaerobic Digesters And Co-Digesters*. Retrieved from <http://www.calepa.ca.gov/Digester/Documents/GuideDigester.pdf>
- CARB. (2015). Climate Change Programs. Retrieved March 12, 2015, from <http://www.arb.ca.gov/cc/cc.htm>
- CEC. (2015). Anaerobic Digestion. Retrieved April 12, 2015, from <http://www.energy.ca.gov/biomass/anaerobic.html>
- Chang, J. S., Lee, K. S., & Lin, P. J. (2002). Biohydrogen production with fixed-bed bioreactors. *International Journal of Hydrogen Energy*, 27(11-12), 1167–1174. [http://doi.org/10.1016/S0360-3199\(02\)00130-1](http://doi.org/10.1016/S0360-3199(02)00130-1)
- Choudhuri, A. R., & Gollahalli, S. R. (2000). Combustion characteristics of hydrogen - hydrocarbon hybrid fuels. *International Journal of Hydrogen Energy*, 25, 451–462. Retrieved from <http://www.sciencedirect.com/science/article/pii/S0360319999000270#>
- Collier, K., Hoekstra, R. L., Mulligan, N., Jones, C., & Hahn, D. (1996). *Untreated Exhaust Emissions of a Hydrogen-Enriched CNG Production Engine Conversion*. Retrieved from <http://papers.sae.org/960858/>
- Cooney, M., Maynard, N., Cannizzaro, C., & Benemann, J. (2007). Two-phase anaerobic digestion for production of hydrogen – methane mixtures, 98, 2641–2651. <http://doi.org/10.1016/j.biortech.2006.09.054>

- Crookes, R. J. (2006). Comparative bio-fuel performance in internal combustion engines. *Biomass and Bioenergy*, 30(5), 461–468. <http://doi.org/10.1016/j.biombioe.2005.11.022>
- Das, D., Khanna, N., & Dasgupta, C. N. (2014). *Biohydrogen Production: Fundamentals and Technology Advances*. CRC Press. Retrieved from https://books.google.com/books?id=sXvNBQAAQBAJ&pg=PA240&lpg=PA240&dq=reducing+hydrogen+partial+pressure+by+stirring&source=bl&ots=y2OHxohsD-&sig=pnrGtSHZ1P5HtLCWyet_8_ce-7E&hl=en&sa=X&ei=Sm5LVaWBEsm0ggTkzoCwBg&ved=0CCIQ6AEwAQ#v=onepage&q=reducing hydrogen
- Demirel, B., & Yenigün, O. (2002). Two-phase anerobic digestion processes: a review. *Journal of Chemical Technology & Biotechnology*, 77(7).
- DOE. (2004). *DOE Workshop on Hydrogen Production Via Direct Fermentation*. Retrieved from https://www1.eere.energy.gov/hydrogenandfuelcells/pdfs/fermentation_wkshp.pdf
- DOE. (2013). Anaerobic Digestion Basics. Retrieved March 12, 2015, from <http://energy.gov/eere/energybasics/articles/anaerobic-digestion-basics>
- DOE. (2014). Alternative Fuels Data Center: Hydrogen Production and Distribution. Retrieved January 1, 2015, from http://www.afdc.energy.gov/fuels/hydrogen_production.html
- Donkin, S. S., Koser, S. L., White, H. M., Doane, P. H., & Cecava, M. J. (2009). Feeding value of glycerol as a replacement for corn grain in rations fed to lactating dairy cows. *Journal of Dairy Science*, 92(10), 5111–5119. <http://doi.org/10.3168/jds.2009-2201>
- Dürre, P. (2005). *Handbook on Clostridia*. Boca Raton, FL: CRC Press.
- Effendi, a., Hellgardt, K., Zhang, Z. G., & Yoshida, T. (2005). Optimising H₂ production from model biogas via combined steam reforming and CO shift reactions. *Fuel*, 84(7-8), 869–874. <http://doi.org/10.1016/j.fuel.2004.12.011>
- ESA. (2011). *Economic Feasibility of Dairy Manure Digester and Co-digester Facilities in the Central Valley of California*. Retrieved from http://www.waterboards.ca.gov/centralvalley/water_issues/dairies/dairy_program_regs_requirements/final_dairy_digstr_econ_rpt.pdf
- Fang, H. H. P., & Liu, H. (2002). Effect of pH on hydrogen production from glucose by a mixed culture. *Bioresource Technology*, 82(1), 87–93. [http://doi.org/10.1016/S0960-8524\(01\)00110-9](http://doi.org/10.1016/S0960-8524(01)00110-9)
- Fresco, E. (2015). *Digestion of Microalgae for Methane Production and Nutrient Recycling*.

- FSEC. (2014). Hydrogen Basics - Production. Retrieved January 1, 2015, from <http://www.fsec.ucf.edu/en/consumer/hydrogen/basics/production.htm>
- Gavala, H. N., Skiadas, I. V., & Ahring, B. K. (2006). Biological hydrogen production in suspended and attached growth anaerobic reactor systems. *International Journal of Hydrogen Energy*, 31(9), 1164–1175. <http://doi.org/10.1016/j.ijhydene.2005.09.009>
- Ghosh, S., Ombregt, J. P., & Pipyn, P. (1985). Methane production from industrial wastes by two-phase anaerobic digestion. *Water Research*, 19(9), 1083–1088. [http://doi.org/10.1016/0043-1354\(85\)90343-4](http://doi.org/10.1016/0043-1354(85)90343-4)
- Hallenbeck, P. C., & Benemann, J. R. (2002). Biological hydrogen production; Fundamentals and limiting processes. *International Journal of Hydrogen Energy*, 27(11-12), 1185–1193. [http://doi.org/10.1016/S0360-3199\(02\)00131-3](http://doi.org/10.1016/S0360-3199(02)00131-3)
- Hill, A. (2014). *Effect of Pretreatment Technologies of Wastewater Grown Microalgae: In Preparation for Anaerobic Digestion*.
- Holladay, J. D., Hu, J., King, D. L., & Wang, Y. (2009). An overview of hydrogen production technologies. *Catalysis Today*, 139(4), 244–260. <http://doi.org/10.1016/j.cattod.2008.08.039>
- Jones, G. M. (2008). *Pumping Station Design*. (G. Tchobanoglous, B. E. Bosserman, & R. L. Sanks, Eds.). Retrieved from [https://books.google.com/books?id=biWHfrpd9gsC&pg=SA14-PA6&lpg=SA14-PA6&dq=catalytic+converters+fouled+by+biogas&source=bl&ots=wNcNkojBCv&sig=jty87CPoVPMQVwxYhR_hdGMZNt0&hl=en&sa=X&ved=0ahUKEwi5wLiyj9fJAhUxIIMKHVWYAI4Q6AEIJDAB#v=onepage&q=catalytic converters fouled by biogas&f=false](https://books.google.com/books?id=biWHfrpd9gsC&pg=SA14-PA6&lpg=SA14-PA6&dq=catalytic+converters+fouled+by+biogas&source=bl&ots=wNcNkojBCv&sig=jty87CPoVPMQVwxYhR_hdGMZNt0&hl=en&sa=X&ved=0ahUKEwi5wLiyj9fJAhUxIIMKHVWYAI4Q6AEIJDAB#v=onepage&q=catalytic%20converters%20fouled%20by%20biogas&f=false)
- Karakashev, D., & Angelidaki, I. (2011). *Chapter 23: Thermophilic Biohydrogen Production, of Biofuels: Alternative Feedstocks and Conversion Processes*. Elsevier. Retrieved from [https://books.google.com/books?id=DnyfnOsTLfcC&pg=PA526&lpg=PA526&dq=stoichiometric+glucose+to+hydrogen+12+mol/mol&source=bl&ots=TfzbNHATQY&sig=QPOOeoPtqASXpo-FhM1LAQAY0Vg&hl=en&sa=X&ei=-803VYqDJ8uYgwSt9oHgAw&ved=0CDMQ6AEwBQ#v=onepage&q=stoichiometric gl&](https://books.google.com/books?id=DnyfnOsTLfcC&pg=PA526&lpg=PA526&dq=stoichiometric+glucose+to+hydrogen+12+mol/mol&source=bl&ots=TfzbNHATQY&sig=QPOOeoPtqASXpo-FhM1LAQAY0Vg&hl=en&sa=X&ei=-803VYqDJ8uYgwSt9oHgAw&ved=0CDMQ6AEwBQ#v=onepage&q=stoichiometric%20gl&)
- Kim, S. H., Han, S. K., & Shin, H. S. (2006). Effect of substrate concentration on hydrogen production and 16S rDNA-based analysis of the microbial community in a continuous fermenter. *Process Biochemistry*, 41(1), 199–207. <http://doi.org/10.1016/j.procbio.2005.06.013>
- Kornbluth, K., Greenwood, J., Jordan, E., McCaffrey, Z., & Erickson, P. a. (2012). Economic feasibility of hydrogen enrichment for reducing NOx emissions from landfill gas power generation alternatives: A comparison of the levelized cost of electricity with present strategies. *Energy Policy*, 41(x), 333–339. <http://doi.org/10.1016/j.enpol.2011.10.054>

- Krich, K., Augenstein, D., Batmale, J., Benemann, J., Rutledge, B., & Salour, D. (2005). *Biomethane from Dairy Waste: A Sourcebook for the Production and Use of Renewable Natural Gas in California*. Retrieved from <http://suscon.org/cowpower/biomethaneSourcebook/biomethanesourcebook.php>
- Liang, K., & Pirnie, M. (2009). Regulatory Impacts of Biogas-fired Internal Combustion Engines. In *CWEA Air, Water, & Energy Conference: Sustainability for Wastewater Treatment Plants*. Retrieved from <https://www.cwea.org/sarbs/pdfs/AirWaterEnergyConf/BiogasEngineRegCWEAJune112009.pdf>
- Lin, C. Y., Lee, C. Y., Tseng, I. C., & Shiao, I. Z. (2006). Biohydrogen production from sucrose using base-enriched anaerobic mixed microflora. *Process Biochemistry*, 41(4), 915–919. <http://doi.org/10.1016/j.procbio.2005.10.010>
- Lin, C.-Y., & Chang, R.-C. (1999). Hydrogen production during the anaerobic acidogenic conversion of glucose. *Journal of Chemical Technology and Biotechnology*, 74(6), 498–500. Retrieved from <http://onlinelibrary.wiley.com/doi/10.1002/%28SICI%291097-4660%28199906%2974:6%3C498::AID-JCTB67%3E3.0.CO;2-D/abstract>
- Lundquist Laboratory, C. P. (2014). *GC Bench Method for Biogas Measurement Using Argon and Helium Carrier Gases*. San Luis Obispo.
- McCarty, P. L. (1964). Anaerobic Waste Treatment Fundamentals. *Public Works*, 95(9,10,11,12). Retrieved from [http://www.seas.ucla.edu/stenstro/Anaerobic assignment.pdf](http://www.seas.ucla.edu/stenstro/Anaerobic%20assignment.pdf)
- Metcalf & Eddy. (2013). *Wastewater Engineering Treatment and Reuse*. New York, NY: McGraw-Hill.
- Mizuno, O., Dinsdale, R., Hawkes, F. R., Hawkes, D. L., & Noike, T. (2000). Enhancement of hydrogen production from glucose by nitrogen gas sparging. *Bioresource Technology*, 73(1), 59–65. [http://doi.org/10.1016/S0960-8524\(99\)00130-3](http://doi.org/10.1016/S0960-8524(99)00130-3)
- Mohan, S. V. (2009). Waste to Renewable Energy. In O. V. Singh & S. P. Harvey (Eds.), *Sustainable Biotechnology: Sources of Renewable Energy*. <http://doi.org/10.1007/978-90-481-3295-9>
- Pettygrove, G. S., Putnam, D. H., & Meyer, D. M. (2003). *Integrating Forage Production with Dairy Manure Management in the San Joaquin Valley*.
- Qorpak. (2015). Flourination: The Solution to Plastic Bottle Paneling. Retrieved July 12, 2015, from <http://www.qorpak.com/pages/Fluorination>
- R-Biopharm. (2015). *UV-method for the Determination of D-glucose in Foodstuffs and Other Materials*. Retrieved from http://www.r-biopharm.com/wp-content/uploads/4050/Glucose_EN_10716251035_2013-03.pdf

- Ruggeri, B., Tommasi, T., & Sanfilippo, S. (2015). *BioH₂ & BioCH₄ Through Anaerobic Digestion: From Research to Full-scale Applications*. Springer-Verlag London.
<http://doi.org/10.1007/978-1-4471-6431-9>
- Shrestha, S. O. B., & Karim, G. . (1999). Hydrogen as an additive to methane for spark ignition engine applications. *International Journal of Hydrogen Energy*, 24(6), 577–586. [http://doi.org/10.1016/S0360-3199\(98\)00103-7](http://doi.org/10.1016/S0360-3199(98)00103-7)
- Sierens, R. (1998). *Variable Composition Hydrogen/Natural Gas Mixtures for Increased Engine Efficiency and Decreased Emissions*. Retrieved from
http://www.althytude.info/fileadmin/user_upload/documents/Sierens-H2NG_mixtures.pdf
- Spierling, R. E., Albinger, L. C., & Lundquist, T. J. (2009). *Projected Performance and Costs of Wastewater Treatment at California Flush Dairies*. San Luis Obispo.
- Sreethawong, T., Niyamapa, T., Neramitsuk, H., Rangsunvigit, P., Leethochawalit, M., & Chavadej, S. (2010). Hydrogen production from glucose-containing wastewater using an anaerobic sequencing batch reactor: Effects of COD loading rate, nitrogen content, and organic acid composition. *Chemical Engineering Journal*, 160(1), 322–332. <http://doi.org/10.1016/j.cej.2010.03.037>
- Sung, S., Raskin, L., Duangmanee, T., Padmasiri, S. I., & Simmons, J. J. (2007). Hydrogen Production by Anaerobic Microbial Communities Exposed to Repeated Heat Treatments. *Water Environment Research : A Research Publication of the Water Environment Federation*, 79(9), 975–83. Retrieved from
<http://www1.eere.energy.gov/hydrogenandfuelcells/pdfs/32405a13.pdf>
- TerMaath, C. Y., Skolnik, E. G., Schefer, R. W., & Keller, J. O. (2006). Emissions reduction benefits from hydrogen addition to midsize gas turbine feedstocks. *International Journal of Hydrogen Energy*, 31(9), 1147–1158.
<http://doi.org/10.1016/j.ijhydene.2005.10.002>
- The White House. (2014). *Climate Action Plan: Strategy to Reduce Methane Emissions*. Washington D.C. Retrieved from
https://www.whitehouse.gov/sites/default/files/strategy_to_reduce_methane_emissions_2014-03-28_final.pdf
- USDA. (2009). *Cooperative Approaches for Implementation of Dairy Manure Digesters. Environmental Protection*. Retrieved from <http://www.rd.usda.gov/files/rr217.pdf>
- USEPA. (2010). California Dairy Digester Workshop. Retrieved May 5, 2015, from
<http://www.epa.gov/agstar/news-events/events/workshop10.html>
- USEPA. (2013). Region 9 Strategic Plan, 2011-14. Geographic Area of Focus: San Joaquin Valley. Retrieved January 1, 2015, from
<http://www.epa.gov/region9/strategicplan/sanjoaquin.html>

- USEPA. (2014a). Climate Change Indicators in the United States: Greenhouse Gases. Retrieved January 5, 2015, from <http://www.epa.gov/climate/climatechange/science/indicators/ghg/index.html>
- USEPA. (2014b). *Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990 - 2012*. Washington, DC. Retrieved from <http://www.epa.gov/climatechange/ghgemissions/usinventoryreport.html>
- USEPA. (2015). Comprehensive Livestock Digester Database. Retrieved from http://www.epa.gov/agstar/downloads/digesters_all_20150129.xlsx
- Valdez-Vazquez, I., & Poggi-Varaldo, H. M. (2009). Hydrogen production by fermentative consortia. *Renewable and Sustainable Energy Reviews*, 13(5), 1000–1013. <http://doi.org/10.1016/j.rser.2008.03.003>
- Wang, G., Mu, Y., & Yu, H.-Q. (2005). Response surface analysis to evaluate the influence of pH, temperature and substrate concentration on the acidogenesis of sucrose-rich wastewater. *Biochemical Engineering Journal*, 23(2), 175–184. <http://doi.org/10.1016/j.bej.2005.01.002>
- Wang, J., & Wan, W. (2009). Factors influencing fermentative hydrogen production: A review. *International Journal of Hydrogen Energy*, 34(2), 799–811. <http://doi.org/10.1016/j.ijhydene.2008.11.015>
- Westermann, P., & Ahring, B. (2006). *Biofuels for Fuel Cells (Integrated Environmental Technology)*. (P. Lens, P. Westermann, & A. Haberbauer, Eds.). IWA Publishing.
- Wilkie, A. (2000). *Fixed Film Anaerobic Digester*. Retrieved from http://www.epa.gov/agstar/documents/Fixed_Film_Anaerobic_Digester.pdf
- Wilson, R. P. (2012). *Application of Hydrogen Assisted Lean Operation to Biogas Fueled Reciprocating Engines (BioHALO)*. Retrieved from <http://www.energy.ca.gov/2012publications/CEC-500-2012-064/CEC-500-2012-064.pdf>
- WtERT. (2009). Anaerobic Digestion Process. Retrieved from <http://www.wtert.eu/default.asp?Menu=13&ShowDok=12>
- Yu, Z., & Schanbacher, F. L. (2009). Production of Methane Biogas as Fuel Through Anaerobic Digestion. In O. V. Singh & S. P. Harvey (Eds.), *Sustainable Biotechnology: Sources of Renewable Energy* (pp. 107–108). Retrieved from https://books.google.com/books?id=TbMWusKqTc8C&pg=PA145&lpg=PA145&dq=2+mol+H2/mol+glucose+practical+limit&source=bl&ots=56JYD-bTP_&sig=N8LQDOCvIU89iC_bND11jcXGzU4&hl=en&sa=X&ei=qNM3VbbvKcK5gGTT4YKgAw&ved=0CCsQ6AEwAg#v=onepage&q=complex community&f=false
- Zhang, Z.-P., Show, K.-Y., Tay, J.-H., Liang, D. T., Lee, D.-J., & Jiang, W.-J. (2006). Effect of hydraulic retention time on biohydrogen production and anaerobic microbial community. *Process Biochemistry*, 41(10), 2118–2123. <http://doi.org/10.1016/j.procbio.2006.05.021>

APPENDICES

Appendix A - Second-Phase Gas Production Rate Calculations

Goal: Calculate the theoretical biogas production rate of the second-phase and compare it to the actual biogas production rate (estimated as the sparging rate) to determine if the second-phase digesters were operating normally.

Known Data: The list below and Table-A1 display relevant data for this calculation:

- Average Feed COD (1st-phase effluent) = $COD_{IN} = 10427.4 \text{ mg/L}$
- Average Effluent COD = $COD_{EFF} = 1218.7 \text{ mg/L}$
- Volume of One Second-phase Digester = $V_{digester} = 20 \text{ L/digester}$
- Total # of Second-phase Digesters = 2
- HRT = 30 days
- Temperature = $T = 30^{\circ}\text{C}$

Table A-1: Gas composition, gas flow, and volumetric H_2 yield for all digesters operated in the integrated experiment.

Digester Name	Avg. Gas Composition			Total Gas Flow (L/day)	Volumetric H_2 Yield (L/day) ³
	% H_2	% CO_2	% CH_4		
Sparged First-phase Digester	25.5	54.5	20.0	9.12 ¹	2.19
Non-Sparged First-phase Digester (Control)	52.7	47.3	0.0	2.46 ¹	1.16
Second-phase Digesters (Combined)	0.0	38.1	61.9	6.66 ²	Does Not Apply

¹ The average gas flow measured by the gas meters during steady-state.

² Gas meters could not be used to directly measure the second-phase production rate. Instead, this value was estimated by subtracting the non-sparged first-phase digester flow rate from the sparged first-phase digester flow rate. This value is approximately equal to 0.28 L gas/L-hr sparging rate.

³ The average of all volumetric H_2 yields calculated for each day at steady state (from Excel spreadsheets). This column may not match the (total gas flow * % H_2).

Solution:

Step 1.) Use COD mass balance to calculate CH_4 -equivalent COD (COD_{CH_4})

$$\begin{aligned}
 COD_{IN} &= COD_{EFF} + COD_{CH_4} \\
 \frac{10427.4 \text{ mg}}{L} &= \frac{1218.7 \text{ mg}}{L} + COD_{CH_4}
 \end{aligned}$$

$$\text{COD}_{\text{CH}_4} = \frac{9208.7 \text{ mg}}{\text{L}}$$

Step 2.) Convert COD_{CH_4} into theoretical volume of CH_4 gas (at 1 atm, $T = 30^\circ\text{C}$) produced per day, per digester (a.k.a. CH_4 production rate)

$$\begin{aligned} \text{Theoretical CH}_4 \text{ Production Rate} &= \text{From Metcalf \& Eddy} \\ &\frac{V_{\text{digester}} (\text{L/digester})}{\text{HRT (day)}} \left(\text{COD}_{\text{CH}_4} \left(\frac{\text{mg COD}}{\text{L}} \right) \right) \left(\frac{1 \text{ g}}{1000 \text{ mg}} \right) \left(\frac{0.39 \text{ L CH}_4}{\text{g COD}} \right) \\ \text{Theoretical CH}_4 \text{ Production Rate} &= \frac{20 \text{ L/digester}}{30 \text{ day}} \left(\frac{9208.7 \text{ mg COD}}{\text{L}} \right) \left(\frac{1 \text{ g}}{1000 \text{ mg}} \right) \left(\frac{0.39 \text{ L CH}_4}{\text{g COD}} \right) \\ \text{Theoretical CH}_4 \text{ Production Rate} &= \frac{2.4 \text{ L CH}_4}{\text{day - digester}} \end{aligned}$$

Step 3.) Calculate the total, combined gas production rate for both digesters assuming 61.9% CH_4 , 38.1% CO_2 in the biogas (from Table A-1)

$$\begin{aligned} \text{Theoretical Gas Production Rate} &= (\text{CH}_4 \text{ Production Rate}) \times (2 \text{ digesters}) \times \left(\frac{100 \text{ L Biogas}}{61.9 \text{ L CH}_4} \right) \\ \text{Theoretical Gas Production Rate} &= \left(\frac{2.4 \text{ L CH}_4}{\text{day - digester}} \right) \times (2 \text{ digesters}) \times \left(\frac{100 \text{ L Biogas}}{61.9 \text{ L CH}_4} \right) \\ \text{Theoretical Gas Production Rate} &= \frac{7.75 \text{ L Biogas}}{\text{day}} \text{ (same as } \frac{0.32 \text{ L Biogas}}{\text{hr}}) \end{aligned}$$

Step 4.) Calculate percent error between theoretical and actual gas production from the second-phase (Note: actual gas production was estimated by assuming the sparging rate was nearly the same value (6.66 L/day or 0.28 L/hr))

$$\% \text{ Error} = \frac{(\text{Theoretical Biogas Production Rate}) - (\text{Actual Biogas Production Rate})}{(\text{Theoretical Biogas Production Rate})} \times 100$$

$$\% \text{ Error} = \frac{7.75 - 6.66}{7.75} \times 100\% = 14.1\%$$