ANAEROBIC FERMENTATION OF FOOD WASTE AND GLYCEROL TO
HYDROGEN

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
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June 2017

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ABSTRACT

Anaerobic Fermentation of Food Waste and Glycerol to Hydrogen

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Hydrogen has several well-known advantages as a fuel and chemical feedstock, but current methods of hydrogen production are costly and energy intensive. A potentially advantageous source of hydrogen is fermentation of organic wastes, especially any abundant, low-cost wastes with a high content of simple sugars. Molar hydrogen yields from fermenters (aka digesters) are affected by pH, organic loading rate (OLR), hydraulic residence time (HRT), and substrate type. A less studied process to increase yield is sparging with low-H₂ content gas to strip H₂ from the digester liquid. The present study optimized the levels of each of these variables for hydrogen production from glycerol and food waste, building on previous proof-of-concept studies that used glucose as the substrate.

Six bench-scale, semi-continuously fed, stirred, anaerobic digesters were constructed and fed glycerol or food waste as a substrate. In a series of experiments, pH, HRT, OLR, and gas sparging rate were tested over a range of values. pH levels were controlled by use of phosphate buffers. In an envisioned process, low-H₂ content from a second-stage methane digester would be used as the sparging gas, allowing subsequent combustion of a high-H₂ content biogas with low NO_x formation potential. N₂ was used as a surrogate for biogas in one set of experiments.

The main conclusions are based on data from periods of steady-state digester performance and daily measurements of pH, alkalinity, biogas production, biogas composition, total and volatile suspended solids, and chemical oxygen demand (COD). COD balances were measured for all experiments and generally showed recoveries of >85%.

With glycerol substrate, the highest molar hydrogen yield (0.071 ± 0.0100 mol H_2 /mol glycerol) and volumetric hydrogen production (0.281 ± 0.0395 $L_{H_2}/L_{Reactor}$ -day) were achieved with the following: pH 6.51, OLR 18.8 g COD/L-day, HRT 12 hours, and sparging rate of 3.2 mL/min, and 1-L working volume. Gas type (N_2 or biogas) used in sparging did not influence hydrogen production.

The best results with food waste (0.021 ± 0.0013 mol H_2 /mol COD and 0.478 ± 0.0280 L $H_2/L_{Reactor}$ -day) were obtained with the following conditions: OLR 33.9 g COD/L-day and nitrogen sparging rate of 1.0 L N_2 /hour, and 1-L working volume. pH and HRT were not optimized for food waste substrate, but the best values from the glycerol experiments were adopted.

Sparged glycerol and food waste digesters had molar hydrogen yields at least 40% greater than controls. Nonetheless, molar hydrogen yields in the present study were lower than in those reported by other authors, for unknown reasons. Yields from food waste might be improved by optimizing pH and HRT

levels. Alkalinity sources need to be identified to replace the non-scalable phosphate buffers of the present research. Lastly, long-term experiments should consider whether attached growth of hydrogen-consuming methanogens develops in hydrogen fermentation reactors.

ACKNOWLEDGMENTS

I would like to thank,

Carol and Jacques Krikorian for working two jobs, and sacrificing so much for me to get an excellent education and further my dreams, for teaching me the values of a hard work ethic, and responsibility, and for supporting me and my endeavors every step of the way

Tyler Deitz for putting up with an irritated Eric whose just spent hours and hours in a stinky lab, for being someone I look forward to seeing every single day, for somehow making even the worst of situation better, and for helping me to become a better person every day

Ruth Spierling for two years of answering my relentless phone calls and emails, supporting me every step of the way, teaching me everything I know about the world of anaerobic digestion, and inspiring me every day to become a better scientist and engineer

Tryg Lundquist for his ability to make even the most scientific of discussions humorous, and for inspiring me to work harder and harder every day to pursue one of the most important and extraordinary fields of engineering

Shelley Blackwell for her support in scheduling ENVE 405 students and taking care of my many emergency shipping orders

Christopher O'Connor, Emma Hennig, and Daniel Covarrubias for their hours of hard work and support in the lab, and for allowing me to have an occasional weekend

ENVE 405 students, for collecting much of my data

Rebekah Oulton for being in my thesis committee and teaching me the value of project management

Chris Kitts for being in my thesis committee

California Energy Commission for financial support in a project grant through the Energy Innovation Small Grants program

Tracy Thatcher for lending me the air flow meter

San Luis Obispo Water Reclamation Facility for allowing me to pick up gallons and gallons of sludge

California Polytechnic State University, San Luis Obispo for allowing me to learn
about the joys of anaerobic digestion

And everyone else who was instrumental to my success in this project

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CHAPTER 1: INTRODUCTION

The future of energy production lies not in the use of fossil fuels, rather the more sustainable use of renewable energy including renewable hydrogen. Hydrogen energy is expected to become one of the dominant sources of energy due to its abundance of environmental applications, high energy yield (142.3 kJ/g), and formation of water as its only combustion product (Seifert, Waligorska, Wojowski, & Laniecki, 2009). Hydrogen is a promising alternative to fossil fuels because the energy yield (kJ/g) of hydrogen is 2.75 times higher than that of traditional fossil fuels (Maru, Bielen, Constanti, Medina, & Kengen, 2013, Han et al., 2016, Sharma, Parnes, & Li, 2011). The microbial conversion of organic waste substrates has proven itself as a promising means of producing hydrogen gas (Lo, Chen, Huang, Yuan, & Chang, 2013; Seifert et al., 2009).

Hydrogen use is currently centered around its many industrial applications like refining, metallurgy, and electronics (Ramachandran & Menon, 1998). However, its use as a source of energy is slowly gaining ground, especially in applications like hydrogen fuel cells and fuel hydrogenation. Hydrogen fuel cells are being studied extensively as an option for transportation systems because they do not produce greenhouse gasses, and their energy conversion efficiency is generally greater than the Carnot efficiency limit of traditional internal combustion engines (Ahmadi & Kjeang, 2017). Concurrently, a series of hydrogen refueling stations along what is being called the “California Hydrogen Highway” is being considered to support the future of transportation (Romm, 2006). Hydrogen gas is also being

studied as a possible additive to biogas in biogas energy generators. Hydrogen has been shown to reduce harmful pollutants like nitrogen oxides when mixed with biogas and combusted in lean air to fuel ratios (Liang & Pirnie, 2009; Wilson, 2012; Choudhuri & Gollahalli, 2000). Even so, hydrogen storage, transportation, and production issues have presented difficulties for implementation (Kornbluth, Greenwood, Jordan, McCaffery, & Erickson, 2012). Ahmadi and Kjeang note the lack of hydrogen production infrastructure as being the major drawback to its potential energy uses (2017).

Hydrogen is most commonly produced by means of steam reforming natural gas, thermochemical and radiolytic processes, and water electrolysis (Department of Energy, 2014; Maru et al., 2013). However steam reforming is three times more expensive per energy unit than gasoline, and water electrolysis is only feasible in areas where electricity is inexpensive (Florida Solar Energy Center, 2014). Most importantly, electro- and thermo-chemical hydrogen production is dependent on fossil fuel energy, and the means of production are energy inefficient (Maru et al., 2013). Fermentative hydrogen production, or the anaerobic digestion of organic waste substrates to hydrogen, may pose a solution to these problems by utilizing natural, low energy input microbial processes (Chong et. al. 2008, Tapia-Venegas et al., 2015).

Fermentative hydrogen production has many benefits over traditional and non-microbial forms of hydrogen production. First, fermentative hydrogen production can use carbon-rich and abundant wastes like glycerol and food waste to

produce hydrogen gas. Rather than being sent to the landfill, these wastes can be used for hydrogen production, and in the process become stabilized and minimized before being disposed of or used in other potential applications (Kumar et al., 2017). If produced on site with existing methanogenic digesters, hydrogen digesters can work in series with the methanogenic digesters, creating a two-phase system resulting in hydrogen and methane gas mixtures for combustion. Furthermore, fermentative hydrogen production can be a low-cost and environmentally friendly process when organic waste substrates are used.

One of the most important factors in producing hydrogen by microbial processes is the substrate fed to the microorganisms. For substrates to be feasible for fermentative hydrogen production, they must be simple sugars, low-cost, present in large quantities, and the nature of carbon in the substrate must be highly reduced (Maru et al., 2013). Glycerol and food waste are two wastes that meet these criteria. However, a large amount of hydrogen research uses glucose substrate. Glucose is the ideal substrate for hydrogen production, and it is good for demonstrating the process, but it is bad for real world applications because it is not a waste.

Glycerol is produced, in large amounts, during the transesterification of vegetable oils, and animal fats for biodiesel and bioethanol production. For every 100 pounds of biodiesel produced, roughly 10 pounds of crude glycerol is produced (Yazdani & Gonzalez, 2007). Due to the accelerated growth of biodiesel and bioethanol industries, glycerol is being produced in surplus, resulting in a 10-fold

decrease in glycerol prices (Maru et al., 2013; Yazdani & Gonzalez, 2007)

Because of the highly-reduced carbon in glycerol, microbial fermentation to more valued products like 1,3-propandiol, ethanol, acetic and butyric acid, and hydrogen makes glycerol a promising substrate for fermentative hydrogen production (Yazdani & Gonzalez, 2007).

Stoichiometrically, 1 mole of hydrogen can be produced per mole of glycerol consumed (Equation 1-1). However, actual hydrogen yields are expected to be much less than the stoichiometry indicates due to reactions that produce other desired compounds (Yazdani & Gonzalez, 2007; Hallenbeck & Benemann, 2002). Even if the reactions were controlled in a way to produce only hydrogen and ethanol, heat losses would prevent the reaction from reaching full stoichiometric yields.

Every year the United States disposes 32.2 million tons of food waste, or about 0.279 kg per person, per day (Krista, Tonjes, Gurevitch, 2015). Food waste is present in large quantities, and accounts for roughly 40% of municipal solid waste (Han et al., 2016). Food waste varies by source, but is generally comprised of simple sugars, fats, carbohydrates, and proteins - compounds that have a great potential for hydrogen production (Curry & Pillay, 2012). One source found the hydrogen production potential of post-consumer food waste mixed with 3% wastewater sludge to be 121.6 mL/g carbohydrate COD (Kim, Han, & Shin, 2004).

One of the drawbacks to fermentative hydrogen production is that there are no accepted and commercialized means to producing hydrogen because of an issue of low molar hydrogen yields. This is an issue if the substrates are not wastes, but if organic waste substrates are used and are available at low or no cost, then the molar yield should be less important. Most experiments test fermentative hydrogen production in batch reactors. However, further examination of continuously stirred tank reactors with organic waste substrates is needed.

Operational conditions including pH, organic loading rate, hydraulic residence time and gas sparging have been found to significantly affect hydrogen production in CSTR anaerobic digesters (Pakarinen, Kaparaju, & Rintala, 2011; Olivas, 2015). Two useful output metrics for determining the optimal conditions, especially for biogas studies, are molar fuel yield and volumetric fuel production. Molar hydrogen yield is a useful output metric because it allows for the comparison of hydrogen yields from different substrates. It can also be a measure of substrate utilization efficiency, a way to determine how efficiently substrates are converted to desired products. The molar hydrogen yield is the moles of hydrogen gas produced by the digesters per mole of substrate COD introduced ($\text{mol H}_2/\text{mol O}_2$). For pure substrates, molar hydrogen yields can be expressed as moles of hydrogen per mole of substrate. This allows for the comparison to stoichiometric yields. While stoichiometric yields are not practical to achieve in biological systems, they are a good benchmark (Hallenbeck & Benemann, 2002). The volumetric hydrogen production is the volume of

hydrogen produced per time per liquid volume in the digester ($L\ H_2/L_{\text{Reactor}}\text{-day}$). Volumetric hydrogen production is the volume of hydrogen produced per volume of digester which is proportional to the capital cost.

The anaerobic digestion process consists of multiple stages where substrate is degraded to other products: (1) hydrolysis, (2) fermentation, (3) acetogenesis, (4) methanogenesis (Cooke, 2014). In fermentative hydrogen production, anaerobic digesters are operated in a way that prevents methanogenesis from occurring. The methanogenic bacteria work against hydrogen production and reduce hydrogen yields by utilizing hydrogen to produce methane (Gunaseelan, 1997; Nallathamb, Thompson, 2008). In methane-producing digesters, the methanogenic bacteria flourish within a pH range of 6.6-7.6, yet they can grow and survive at lower pH values (McCarty, 1964). Hydrogen-producing bacteria, in contrast, typically thrive within a pH range of 5.0-6.5 (Valdez-Vazquez & Poggi-Varaldo, 2009). Consequently, pH can be a key parameter in optimizing hydrogen production.

The hydraulic residence time (HRT) of a CSTR is modeled as the volume of the reactor over the volumetric flow rate. The HRT of hydrogen-producing anaerobic digesters is generally a matter of hours, while for methane-producing digesters, HRT is usually days or weeks (Kuruti et al., 2017). As a rough comparison, the specific growth rate of hydrogen-producing bacteria is 0.215/hr, while methanogenic bacteria have a specific growth rate that is 4-times lower, 0.05/hr (Ruggeri, Tommasi, & Sanfilippo, 2015). Due to their faster growth rate,

hydrogen-producing bacteria can be selected over methanogens by operating with an HRT that is short enough to wash-out the methanogenic bacteria.

Further, methanogen inhibition can occur at lower HRTs due to the accumulation of volatile fatty acids which cause a decrease in the pH of the reactor (Valdez-Vazquez & Poggi-Varaldo, 2009).

The organic loading rate (OLR) is the mass of substrate entering the digester per volume of digester per day (g COD/L-day). Substrate is typically expressed in terms of COD as both pure and unidentified substrates can be expressed as COD. Past studies have achieved molar hydrogen yields ranging from 0.38-0.50 mol H₂/mol glycerol at OLRs ranging from 12.2 g COD/L-day to 24.3 g COD/L-day. Food waste OLRs ranging from 28 g COD/L-day to 50 g COD/L-day have produced molar hydrogen yields of 0.04-0.05 mol H₂/mol COD (Li et. al. 2008b, Lee et. al. 2010a).

High partial pressures of hydrogen inside anaerobic digesters result in the dissolution of gases into the liquid phase, reducing overall hydrogen production and substrate conversion efficiency (Beckers et al., 2015). To reduce the partial pressure of hydrogen inside the digesters, and thus release the gas from the liquid phase, the digesters can be mixed and sparged with an inert gas (Das, Khanna, & Dasgupta, 2014; Beckers et al., 2015). Lamed, Lobos, and Su found that the dissolved hydrogen concentration in liquid digestate was decreased three-fold when mixed, suggesting a three-fold increase in overall hydrogen production (1988). A continuously stirred anaerobic digester fed glucose

increased its molar hydrogen yield from 0.85 mol H₂/ mol glucose to 1.43 mol H₂/ mol glucose when sparged with nitrogen gas at 2.9 L N₂/L-hr (Mizuno, Dinsdale, Hawkes, Hawkes, & Noike, 2000).

With future process feasibility in mind, these experiments are an attempt to address many of the problems facing fermentative hydrogen production. First, continuously stirred tank reactors, rather than batch reactors, were used to perform digestion experiments. Low molar hydrogen yields will be addressed by determining the optimal pH, HRT, OLR, and gas sparging rates. Finally, the use of waste substrates, food waste and glycerol, will be studied in an attempt to determine their feasibility.

CHAPTER 2: METHODS

Laboratory digesters were used in this study to determine the optimal operational conditions for producing hydrogen in semi-continuously fed, stirred anaerobic digesters, as described in detail below. An unusual aspect of the work, and a major objective, was the effort to increase hydrogen yields by stripping hydrogen from the digester liquid phase by sparging with nitrogen gas or biogas.

2.1 Experimental Concept

Bench-scale, semi-continuously fed, stirred, anaerobic digesters were fed glycerol or food waste as a substrate. Hydrogen production was optimized for individual variables: culture pH, hydraulic residence time (HRT), organic loading rate (OLR), or gas sparging rates. Hydrogen production was expressed as either molar or volumetric hydrogen production.

Digester pH levels were controlled by phosphate buffer solutions mixed into the digester feedstock and by automatic pH-stat pumping of a base solution into the digesters. The HRT (Equation 2-1) was controlled by pumps, and OLRs (Equation 2-2) were set by the substrate concentration selected, for the given HRT.

$$\text{Hydraulic Residence Time} = \frac{\text{Working Volume of the Digester (L)}}{\text{Volumetric flowrate in/out of digester (L/t)}} \quad (\text{Eq. 2-1})$$

$$\text{Organic Loading Rate} = \frac{\text{Concentration of Substrate in the Feedstock (g/L)}}{\text{Hydraulic Residence Time (t)}} \quad (\text{Eq. 2-2})$$

Each variable was tested over a range of values in separate experiments. For example, when the optimum pH was being determined, all other variables were held constant. When the optimum pH was found, it was then adopted and held constant while the next variable was tested. Optimal conditions were those that produced the highest molar hydrogen yield or volumetric hydrogen production. Lastly, a range of sparging rates was tested to find the optimal sparging rates for hydrogen production with glycerol or food waste substrate (Table 1). A problem with this stepwise approach was that the operational variables were dependent, meaning that the optimal pH might be different for different HRT and OLR values. The benefit to this one-at-a-time optimization method was that it was straightforward and clearly revealed the impacts of each operational variable on hydrogen yields and production.

Table 1. Operational variables, either treatment variables or constant variables, for each experiment. The stepwise optimization experiments were conducted in the order listed.

Treatment Variable	Operational Variables
pH	Constants: Hydraulic Residence Time, Organic Loading Rate, No Sparging
Organic Loading Rate	Constants: Hydraulic Residence Time, Optimal pH, No Sparging
Hydraulic Residence Time	Constants: Optimal Organic Loading Rate, Optimal pH, No Sparging
Sparging Rate	Constants: Optimal Organic Loading Rate, Optimal pH, Optimal Hydraulic Residence Time

In experiments with glycerol as feed, pure glycerol was used to avoid the potentially inhibitory compounds in crude glycerol from biodiesel production. For food waste feeding experiments, ~91 kilograms of post-consumer food waste was collected from The Avenue, a campus dining hall at California Polytechnic State University, San Luis Obispo.

2.2 Bench-Scale Anaerobic Digesters Design

Six hydrogen fermentation digesters were constructed and operated in duplicate, so three levels of each operational variable were tested simultaneously during each experiment. Each digester vessel was a 2-L bottle with 1.3-mm thick walls of fluorinated high-density polyethylene (FLPE) (Nalgene, ThermoFisher Scientific, Waltham, Massachusetts). FLPE was selected because of its low permeability to hydrogen gas.

Holes were drilled in each vessel for a temperature probe, pH probe, inlet and outlet ports, gas sparging port, gas exit port, and base addition port (Figure 1 & 2). Holes were fitted with 6.4-mm inner diameter (ID) barbed bulkhead fittings (Nalgene, ThermoFisher Scientific) and sealed with Lexel adhesive caulk (Sashco, Brighton, Colorado). Tubing, 6.4-mm ID, (Masterflex Tygon E, Cole-Parmer, Vernon Hills, Illinois) was connected to the bulkhead fittings inside the digester for the inlet, outlet, and sparging ports to a depth of half of the liquid volume. Connected to the sparging tubing was a 20-cm diameter, oval air stone (Uxcell, Hong Kong) for use in sparging experiments.

The temperature port was constructed with a 12.7-mm ID Nalgene barbed bulkhead fitting the inside of the digester vessel, sealed at the bottom using zip ties and Lexel adhesive caulking. Sealing the end of this tube provided for a dead end inside the digester, allowing it to be filled with deionized water from outside the digester for more accurate temperature readings and control. A 6.4-mm compression fitting with a 12.7-mm female threaded adapter (Parker Hannifin,

Cleveland, Ohio) was screwed onto a 12.7-mm male threaded nipple (Cole-Parmer) for the airtight enclosure of the pH probe. All digesters were equipped with a MC122 pH controller, a MP810 dosing pump (both from Milwaukee Instruments, Rocky Mount, North Carolina), and an Extra-Long 220- x 6-mm pH electrode (Cole-Parmer) calibrated at the start of each experiment.

Figure 1. Schematic cross section of a typical digester used in the hydrogen optimization experiments. The lower end of the temperature port tube was sealed and filled with water. A temperature probe was sealed in this tube.

Figure 2. Digester inlets and outlets. Pictured at 1 o'clock is the gas outlet, 3 o'clock is the compression fitting and pH probe, 5 o'clock is the digestate outlet, 7 o'clock is the feed inlet, 9 o'clock is the sealed temperature probe, and 11 o'clock is the base addition line. Pictured in the center is the sealed lid and sparging gas inlet fitting. Also shown is the reflective heat mat wrapped around the digester.

Jumpstart Seedling Heat Mats (Hydrofarm, Petaluma, California) were affixed to duct insulation, wrapped around the digesters, and fastened with Velcro (Carlstadt, New Jersey). Heat mats were connected to Jumpstart Digital Temperature Controllers (Hydrofarm), and temperature probes were inserted into the temperature port and filled with deionized water. Temperature ports were capped with a rubber fitting and periodically filled with deionized water when low. The digesters were held at a constant $35 \pm 2.0^{\circ}\text{C}$.

Gas generated in the headspace of each digester passed through the gas outlet fitting, which was connected to 6.4-mm ID Masterflex Tygon E-Lab tubing. The outlet tubing included a T-fitting with a compression fitting (Cole-Parmer) holding a septa for gas sampling (Thermo Fisher Scientific), another T-fitting leading to a

0.5-L tedlar bag (Zefon, Ocala, Florida) to buffer gas flow, and a 6.4-mm one-way check valve (Cole-Parmer). The gas outlet tubing terminated at a tipping gas meter (Figure 3).

Figure 3. Outlet gas appurtenances

The gas meters contained two triangular-prism chambers in a tipping device, and were submerged in 13 cm of water. When one chamber would fill with enough gas to cause a tip, magnets attached to the tipping mechanism would trigger a reed switch (Standex-Meder Electronics, Cincinnati, Ohio) to open or close (Figure 4). The signal from the reed switch, as well as a timestamp, was recorded on a HOBO 4-Channel Pulse Data Logger (Onset, Bourne, Massachusetts). The meters were calibrated by injecting air through a dry gas flow meter. The average tip volume determined during calibration was 100 ± 9.0 mL. Gas production was calculated as follows:

Daily Gas Production =
Number of Tips per Day ×
(Eq 2-3)

Average Tipping Volume

Figure 4. One of the tipping gas meters (not filled with water). Top: plan view. Bottom: side view.

Two peristaltic pumps (Masterflex L/S, HV-07522-20, Cole-Parmer) delivered feedstock to the six digesters, and two additional pumps removed digestate from the digesters. The pumps' internal program allowed flow rates of 0.001 to 3400 mL/min and multiple start-stop times per day. Masterflex L/S peristaltic pumps were also used to deliver gas into the digesters during the sparging experiments.

During the sparging experiments, sparging gas for each digester was held in a 25-L Tedlar gas bag. The sparging gas was either high purity nitrogen or biogas consisting primarily of methane and carbon dioxide. Masterflex Tygon E-Lab tubing was connected to each of the four bags, routed through the peristaltic pump, and attached to the N₂/CH₄ inlet port of four of the six digesters. When the volume of gas inside of the bags was low, bags were flushed with their respective sparging gas three times, and refilled.

2.2.1 Leak Testing of Digesters

Gas leak testing was performed on each digester before the start of each experiment. First, the digester ports were closed, and the digesters were pressurized with nitrogen gas to 41 kPa (6 psi) and submerged into a sink filled with water. If, after one minute, no bubbles were observed, the digester was deemed ready for a 12-hour leak test. The 12-hour leak test involved filling the digester with one liter of water, closing all digester openings, and connecting a 60-cm tall column of water to the digester inlet port. The digester headspace was pressurized by the 60-cm tall column of water, and the level on the water column

was noted. If the water level in the column did not change over the 12-hour period, the reactor was deemed leak-proof and used in experiments.

On a few occasions, leaks were suspected during experiments. Leaks were identified by increasing concentrations of nitrogen in the biogas, and a decrease in biogas production. The decrease in biogas production lowered the pressure inside of the digester and allowed ambient air to infiltrate the digester. When a leak was suspected, all ports were re-sealed with Lexel adhesive caulking. If the leak could not be stopped, the digester liquid contents were collected in a container, sparged with nitrogen to produce anaerobic conditions, and stored in a 35°C incubator until the digester was repaired and leak tested.

2.2.2 Feedstock Reservoir Design

Each duplicate pair of digesters was fed from one feedstock reservoir (10-L FLPE carboys, Nalgene, Thermo Fisher Scientific). Two holes were drilled at the bottom of each carboy and fitted with 6.4-mm Nalgene barbed bulkhead fittings. MasterFlex Tygon E-Lab tubing (ID 6.4-mm) was connected to the fittings and directed through peristaltic pumps into the inlet port on the digesters. Holes were drilled into the lids of the feed reservoirs and fitted with in-line HEPA disk filters (Whatman, GE Healthcare, Chicago, Illinois) to help prevent contamination of the feedstock with airborne microbes. The feedstock reservoirs were placed in a refrigerator at 4°C and mixed with 108-mm cylindrical polytetrafluoroethylene magnetic stir bars (Big Science Inc., Huntersville, North Carolina) and magnetic

stir plates (Figure 5) (MegaMag Genie, Scientific Industries, Bohemia, New York).

Figure 5. One of three filled digester feedstock reservoir atop a stir plate and located in a refrigerator.

2.3 Operations and Maintenance

The following section explains in further detail the setup and loading of the digesters and the methods used to start the operation.

2.3.1 Experimental Startup

Prior to starting a new experiment, digesters and feed reservoirs were disinfected overnight with a bleach solution and then rinsed. Tubing was also bleached, but rinsed immediately.

The inoculum was anaerobically-digested municipal wastewater sludge obtained from the City of San Luis Obispo Water Resource Recovery Facility (WRRF),

which uses trickling filters and nitrifying activated sludge processes. Primary and secondary sludges are thickened and dosed with ferric chloride before digestion in three anaerobic tanks in series. The first two digesters were mixed and operated at 35°C and a 60-day HRT. The third digester was unheated and unmixed with a 16 day HRT, and Digestate from this third digester (1.7% volatile solids content) was the source of inoculum for all the hydrogen optimization experiments.

To start each experiment, fresh inoculum from the WRRF was obtained and filtered through a 4-mm screen to remove particles that might have clogged the tubing. Once filtered, one liter of the inoculum was pumped into each clean, leak-tested lab digester. The digesters were placed onto the stir plates and fitted with the heat mats.

The inoculum was added to the digesters undiluted rather than diluted with substrate, because it allowed the microbes to acclimate to the conditions inside the digester over a longer period of time (~3 HRTs). Anaerobic wastewater sludge was used as the inoculum, rather than a pure hydrogen-producing culture, because of the many disadvantages pure culture systems are faced with on a larger scale.

Digester feedstock was made by mixing substrate, buffer chemicals, 100 mL of nutrient solution and tap water to a final volume of 10 L (Table 2) and placing the mixture in the carboys on magnetic stir plates in a refrigerator at 4°C. The nutrient solution was added to the feedstock reservoirs as sources of vitamins,

minerals, and metals to support microbial growth. Glycerol is not a balanced substrate, so a nutrient solution was added to support the growth of the microbes inside the digesters. Though food waste is more of a balanced substrate, nutrient solution was added for consistency.

Table 2. *Constituents added to the 10-L feedstock reservoirs*

Constituent	Amount Added
Substrate	Varied
Nutrient Solution	100-mL
Na ₂ HPO ₄	Varied
KH ₂ PO ₄	Varied
Tap Water	Varied

Nutrient solution was prepared by obtaining 20 L of wastewater sludge, filtering it through a 4-mm screen, and then autoclaving it at 121°C and 138 kPa gauge (20 psig) for 2 hours. Once cooled, the autoclaved sludge was divided into 100-mL aliquots and stored at -12°C in Ziploc freezer bags. When used, the sludge was thawed and poured into each feedstock reservoir.

The pH probes were calibrated using pH 4 and pH 7 buffers. Air was removed from inlet tubes and the digester headspaces purged with N₂ gas for 3 minutes. For digesters to be sparged, the N₂/CH₄ inlet ports were connected to their respective gas line, otherwise these ports were capped. After the digesters were sealed and anaerobic, the pre-calibrated and programmed peristaltic pumps were started to initiate operation.

2.3.2 Daily Maintenance

Each day, the feed and effluent tubes were checked for clogging and gas traps and the digesters and feedstock reservoirs were checked to ensure mixing. The liquid volume of each digester was recorded and adjusted by adding feedstock if not at 1 L. Temperature ports were filled with water if low. Potassium hydroxide containers for pH correction were filled. Gas meters were also filled with water if below the calibration level. When the feedstock was low, feedstock reservoirs were removed from the refrigerator, cleaned with bleach, rinsed with water, and refilled with the feedstock constituents.

2.3.3 Sample Collection

Samples were collected daily from the effluent port of each digester and from the feedstock reservoirs. The effluent port was crimped with a catheter clamp (Graham Field, Atlanta, Georgia) and a 140-mL syringe (Monoject, Kendall, Mansfield, Massachusetts) was connected to the port. Once connected, the catheter clamp was opened, and digestate was withdrawn and pushed back into the digester four times to ensure a representative sample was taken. The effluent port was crimped again, and the syringe with sample was removed and discharged into a 60-mL bottle, with the remainder discharged in a larger 100-mL bottle. The bottles were capped until analyzed, as described below. pH and alkalinity analysis was performed immediately after all samples were taken. Extra feedstock was added to the digesters, as needed, to bring the volume back to one liter.

Preservation of the chemical oxygen demand samples included adding less than 0.25-mL concentrated sulfuric acid to a 50-mL sample until the pH was below 2.0. Samples were then stored at 4°C.

2.3.4 Definition of Steady State Digester Performance

The performance of the digesters while they were in steady-state was more relevant to potential future scale-up than performance during startup. Thus, sample and data analysis were more intensive during steady-state operation. This section describes the physical, chemical, and biological criteria used to identify steady-state periods. Experiments typically ended after at least 5 days of steady state performance.

For the physical criterion, if the digesters were perfect CSTRs, 95% of the inoculum would have washed-out after three hydraulic residence times had passed. In this study, steady-state was defined as possible only after steady operation of four HRTs.

For the chemical criteria, pH and alkalinity values could not be more than 20% different on consecutive days. A difference of 20% was used because it allowed for very minor fluctuations of pH and alkalinity to occur while maintaining relatively consistent performance.

For the biological criterion, gas production (volume per day) could not demonstrate a clear trend. Biogas production was not subject to the 20% rule

because for some conditions, biogas production was so low that the gas meters tipped at long intervals (less than one a day).

2.4 Analytical Methods

Prior to steady-state performance, daily pH and alkalinity were determined for each digestate and feed sample, and gas chromatography was performed for each digester to determine if hydrogen was being produced. Hydrogen production was also measured daily prior to steady state because the data loggers were constantly recording data from the tipping gas meters. However, hydrogen production data prior to the steady state period was not used in calculating molar hydrogen yields or volumetric hydrogen production.

After steady state performance was achieved, pH and alkalinity was measured, gas chromatography was analyzed, and total and volatile suspended solids was measured. Feed and digestate sub-samples were preserved for chemical oxygen demand determination.

The pH and alkalinity were determined according to standard methods (American Public Health Association [APHA], 2005). The pH was measured with a gel type electrode (WD-35801-71, Oakton, Vernon Hills, Illinois) after a 3-point calibration with standard solutions at a pH values of 4, 7, and 10. Alkalinity was measured following Method 2320B by titrating 15 mL of sample with 0.20-N H_2SO_4 to a pH of 4.5. The pH electrode included a temperature probe that provided sample temperature over the course of pH and alkalinity analysis (typically 45 minutes). Any changes in temperature during analysis were noted.

Chemical oxygen demand samples were taken on a daily basis during steady state periods and preserved as described earlier. The closed reflux colorimetric method (Method 5220D, 1997, APHA 2005) was performed with commercially prepared test tubes (CHEMetrics, Midland, Virginia), which were used with a Hach DR/890 Colorimeter (S/N 011090017823 Hach, Colorado) to measure the COD of the samples.

Total and volatile suspended solids were measured daily following standard methods (APHA, 2005). Samples were filtered through 4.7-cm glass fiber filters dried to a constant weight at 105 °C , and then ashed at 550°C.

Biogas composition was determined by gas chromatography (Model 8610, SRI Instruments, Torrance, California). The gas chromatograph (GC) used a thermal conductivity detector and a 1.8-m concentric packed column (Alltech CTR I, Deerfield, Illinois) at 55°C. High purity argon gas was the carrier (310 kPa, 45 psi). Samples of 1 mL were withdrawn from each digester and immediately injected into the GC. Each sample was run for 22 minutes allowing hydrogen, methane, nitrogen, carbon dioxide, and oxygen peaks to be read.

To ensure accuracy of all analytical tests, quality control procedures were used. Splits were performed for each test, while matrix spikes were performed in addition to splits for COD analysis. If the splits were within 10% of each other, they were considered passing. If matrix spikes were used, the recovery was considered passing if within 85% to 115% of the expected concentration.

Samples that did not pass quality control procedures were rerun until passed, or discarded

2.5 Experimental Plan Details

This section provides, in greater detail, the background and methods used to vary the different operational conditions for each experiment performed. Table 3 illustrates the main operational conditions for each experiment.

Table 3. *The various operational conditions and values tested for food waste and glycerol substrates*

Experiment Name	Substrate	Organic Load (g COD/L-day)	HRT (hrs)	pH	Sparging Gas
pH 1	Glycerol	24.32	12	6.2, 6.5, 6.8	None
pH 2	Glycerol	24.32	12	6.2, 6.5, 6.8	None
Organic Loading Rate 1	Glycerol	18, 24, 30	12	6.5	None
Organic Loading Rate 2	Glycerol	12, 18, 24	12	6.5	None
Food waste Organic Loading Rate	Food waste	12.79, 19.18, 25.58	12	6.5	None
HRT 1	Glycerol	18	6, 12, 18	6.5	None
HRT 2	Glycerol	18	3, 6, 9	6.5	None
HRT 3	Glycerol	18	6, 12, 18	6.5	None
Glycerol Sparging	Glycerol	18	12	6.5	Biogas / Nitrogen
Food waste Sparging	Food waste	25.58	12	6.5	Nitrogen

Three different pH values were tested for optimal hydrogen production: 6.2, 6.5, and 6.8. These pH values were achieved in the digesters using phosphate

buffers in the feedstock. The buffer formulations were based on the Henderson-Hasselbach equation.

Feedstocks for the pH experiments (12 hours, 24.32 g COD/L-day) differed only in feed buffer concentrations. pH levels in the digesters were also maintained by the pH monitors which were set to dose in 0.2-M potassium hydroxide (KOH) when the digesters were 0.1 pH units below the set point.

Organic loading rate experiments (6.5, 12 hours) used glycerol or food waste as the substrate. For the glycerol experiments, different amounts of pure glycerol (anhydrous, Carolina Biological, Burlington, North Carolina) were added to the feedstock reservoirs to accomplish organic loading rates ranging from 12 to 30 g COD/L-day. The amount of glycerol to be added to the feedstocks was determined by dividing the desired OLR, in units of g COD/L-day, by the volume of feed added to the digesters in one day. From there, the concentration, in units of g COD/L, was converted to concentration of glycerol knowing that 3.5 moles of oxygen (COD) were required to convert one mole of glycerol (Equation 2-4). Pure glycerol was weighed on a balance and added to feedstock reservoirs.

Post-consumer food waste used in organic loading rate experiments was collected from The Avenue, an on-campus cafeteria at California Polytechnic State University, San Luis Obispo. Food waste is collected at the dining hall by means of separate “composting” trash cans. Roughly 91 kg of fresh food waste

was collected mid-day, and a representative sample of 9.1 kg (10% of the total) was removed and categorized (Figure 6). All 91 kg of food waste was homogenized in an industrial blender (Waring, Conair, East Windsor, New Jersey) in small batches. Each batch of blended food waste was mixed together in a clean 190 liter garbage can, and mixed for consistency. While mixing, portions of blended food waste were removed from the garbage can, poured into 3.75-L Ziploc Freezer bags (S.C. Johnson, Racine, Wisconsin), and stored at -20°C.

Figure 6. Components of food waste (in % wet mass) obtained from a dining hall at California Polytechnic State University, San Luis Obispo, and used as digester feed.

Prior to the start of experiments using food waste as a substrate, food waste bags were thawed, mixed, and filtered through a 4-mm screen to prevent larger pieces from clogging tubing. Representative samples were taken from the filtered food waste and analyzed for total and volatile suspended solids. It was

assumed that the volatile solids concentration was equal to glucose concentration because pure glucose is completely volatilized at 550°C. The glucose concentration was then converted to COD concentration so that it could later be diluted to concentrations corresponding to organic loading rates that were to be tested. Food waste bags were placed back into the freezer until they were ready to be used in the feedstock reservoirs.

Different HRTs were achieved by changing the pumping rate of the influent and effluent peristaltic pumps. Glycerol was used as the substrate for HRT experiments, and the pH and OLR of all of the digesters were held constant. HRT is a factor in calculating OLR, so the concentration of glycerol in the feedstocks differed depending on HRT.

Once the optimal pH, HRT, and OLR were found for glycerol, a sparging experiment was performed on those conditions. Two digesters were sparged with high purity nitrogen gas, two digesters were sparged with biogas from methane-producing anaerobic digesters in the lab, and the final two digesters were unsparged. Various sparging rates were accomplished by changing the flow rate on the peristaltic pump. Different sparging gasses were used to determine whether or not the type of gas had an effect on overall hydrogen production.

The sparging experiment using food waste as the substrate ran under the optimal OLR found in the food waste OLR experiment. pH and HRT experiments were not performed on food waste, so the digesters were run under the optimal conditions found in the glycerol experiments— a pH of 6.5, and an HRT of 12

hours. Four of the six digesters were sparged with high purity nitrogen gas, and two of the digesters were not sparged. Different flowrates were achieved by changing the pumping rate on the peristaltic pump carrying gas to the digesters.

CHAPTER 3: RESULTS

The following section reports the results obtained from each of the individual pH, OLR, HRT, and gas sparging experiments. Data from each experiment was used to produce a chemical oxygen demand balance, and calculate molar hydrogen yields and volumetric hydrogen production.

3.1 pH Experimental Results

The pH experiments were conducted to determine the pH that would maximize hydrogen production for given constant OLRs and HRTs. The optimal OLR and HRT were not yet determined for glycerol, so OLR and HRT values from similar experiments using glucose were used, specifically an OLR of 24.3 g COD/L-day and an HRT of 12 hours (Olivas, 2015).

Two pH experiments were performed and operated at the same conditions to see whether the data obtained was repeatable (Table 4). pH values of 6.2, 6.5, and 6.8 were tested. These pH values were maintained in the digesters for the majority of both experiments; however, fluctuations in pH did occur. pH monitors had an accuracy of ± 0.2 , and the difference in target pH values was ± 0.3 .

Digesters occasionally stopped mixing, causing the pH probes to read pH values that were not representative of the mixed digester. The imprecision of the pH monitors and the occasionally unmixed digester sometimes caused the dosing pumps to activate, adding concentrated KOH when not necessary. When this occurred, the date and pH were recorded, and 100-mL of effluent digestate from the duplicate digester was pumped into the affected digester to reduce the pH. If

the digesters were unable to return to normal operation, the experiment was terminated, and restarted.

Table 4. Target operational variables for pH Experiment 1 and pH Experiment 2

Reactor	pH	OLR (g COD/L-Day)	HRT (hours)
Digester 1 Digester 2	6.20	24.32	12
Digester 3 Digester 4	6.50	24.32	12
Digester 5 Digester 6	6.80	24.32	12

pH Experiment 1 had a steady state period of Days 12-16, for all digesters.

Digester 1 (D1) and Digester 2 (D2) operated at an average pH of 6.08 (Figure 7) and an average alkalinity of 2600 mg CaCO₃/L. D3 and D4 had an average pH of 6.47 and an average alkalinity of 4500 mg CaCO₃/L. D5 and D6 had an average pH of 6.83 and an average alkalinity of 6500 mg CaCO₃/L (Figure 8)

Figure 7. Time-series pH readings for replicate digesters D1 and D2 (target pH 6.2). The mean pH of the duplicate digesters was 6.08 during the steady-state period (within the vertical lines). On Day 3, D2 stopped mixing and was dosed with KOH, raising the pH to 6.50. HRT was 12 hours.

Figure 8. Mean of duplicates pH readings during pH Experiment 1. The steady state period is depicted as the days between the vertical lines.

The HRT was maintained for the entirety of the pH Experiment 1, and the OLR for each set of digesters was within one standard deviation of the target OLR of 24.3 g COD/L-day. D1 and D2 had an average OLR of 25.3 ± 1.00 g COD/L-day (mean $\pm$ SD), D3 and D4 had an average OLR of 25.3 ± 1.16 g COD/L-day, and D5 and D6 had an average OLR of 26.1 ± 1.76 g COD/L-day. The OLR for each digester set was calculated using data obtained from COD analysis of feedstock samples.

pH Experiment 2 was run to ensure the results of pH Experiment 1 were repeatable, and had average pH values of 6.21, 6.47, and 6.83. The steady state period for this experiment occurred on Day 5-9 for D1 and D2, and Day 9-13 for D3-6. pH values during the second experiment ranged from 5.85 to 7.06. The alkalinity increased with pH and averaged 3500 mg CaCO₃/L, 5400 mg CaCO₃/L, and 6600 mg CaCO₃/L for D1-2, D3-4, and D5-6, respectively.

The OLR values for pH Experiment 2 were lower than the target OLR of 24.3 g COD/L-day. D1 and D2 had an average OLR of 20.7 ± 1.57 g COD/L-day (mean $\pm$ SD); D3 and D4 had an average OLR of 22.3 ± 2.08 g COD/L-day; and D5 and D6 had an average OLR of 22.5 ± 1.42 g COD/L-day.

3.1.1 COD Balance

A COD balance was performed for each pH experiment, including influent, effluent, and gaseous COD data during the steady state periods. For a perfectly balanced system, the influent COD mass would be equal to the sum of effluent

and gaseous COD masses for a given period. Influent COD data were measured from feedstock samples, while effluent COD was measured from effluent digester samples. Gaseous COD data were obtained by converting the daily hydrogen and methane gas production data to units of COD. In order to convert hydrogen gas production to COD concentration, the ideal gas law was used to convert the volume of hydrogen gas to moles of hydrogen at room temperature and pressure. The molar amount of hydrogen was then converted to COD concentration by a molar conversion to oxygen (COD). This was repeated for methane gas. Each graph includes the mean influent, effluent, and gaseous COD for each duplicate digester.

During the steady state period of pH Experiment 1, recovery of influent COD in effluent and biogas COD ranged from 92-97% (Figure 9). These high recoveries indicate that the digesters were not leaking biogas and lend credence to the results. The COD recoveries for each experiment are a major factor in judging the level of confidence in the hydrogen production results.

Figure 9. COD balance for pH Experiment 1 where digesters were operated at pH values of 6.08, 6.47, and 6.83, during the steady state performance period. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors ($n = 2$) shown on the digester effluent and biogas bars. The influent standard errors were based on measurements during the steady state performance period ($n = 5$).

pH Experiment 2 was also well balanced (Figure 10). During the steady state period digesters operating a pH of 6.21 with recoveries of 95-99% of influent COD. Again, such high recoveries indicate accurate hydrogen yields.

Figure 10. COD balance for pH Experiment 2 where digesters were operated at pHs of 6.21, 6.54, and 6.84 during the steady state performance period. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors ($n = 2$) shown on the digester effluent and biogas bars. The influent standard errors were based on measurements during the steady state performance period ($n = 5$).

3.1.2 Molar Hydrogen Yields and Volumetric Hydrogen Production

Data were combined from both pH experiments to calculate hydrogen yields and the standard error for digesters running under similar conditions. Molar hydrogen yields and volumetric hydrogen production were calculated over steady state periods. The molar yield was highest (0.013 ± 0.0029 mol H_2 /mol COD) for a mean pH of 6.51 for all four digesters (mean $\pm$ SE). At a mean pH of 6.15, the molar yield was 0.006 ± 0.0010 mol H_2 /mol COD. The highest mean pH tested, 6.84, produced a molar yield of 0.004 ± 0.0014 mol H_2 /mol COD (Figure 11). Despite the high standard error between experiments, digesters operating at a mean pH of 6.51 converted the most moles of COD to hydrogen gas.

Figure 11. Molar hydrogen yields for mean pH values of 6.15, 6.51, and 6.84. The means of steady state performance periods by replicate experiments were used to calculate the standard errors shown on the molar yield bars (n=2). Molar hydrogen yields are based on influent COD.

Volumetric hydrogen production followed a similar pattern. At a pH of 6.51, D3 and D4 produced 0.244 ± 0.0416 L H₂/L_{Reactor}-day (mean $\pm$ SE). D1 and D2 (pH 6.15) produced 0.125 ± 0.0014 L H₂/L_{Reactor}-day, and D5 and D6 produced 0.066 ± 0.0211 L H₂/L_{Reactor}-day (Figure 12). Volumetric hydrogen production for digesters operating at a mean pH of 6.15 produced repeatable results, with only 2.1% difference between experiments. Digesters operating at pH values of 6.51 and 6.84 did not produce repeatable results with molar hydrogen yields between experiments >20% different. A pH of 6.5 was used for the subsequent experiments because it produced hydrogen yields that were greater than the other two pH values tested, and its error was not within the bounds of the other sets of digesters.

Figure 12. Volumetric hydrogen production for mean pH values of 6.15, 6.51, and 6.84. The means of steady state performance periods by replicate experiments were used to calculate the standard errors shown on the volumetric hydrogen production bars (n=2).

3.2 Organic Loading Rate Experiments

The OLR experiments examined the effect of various concentrations of glycerol and food waste on hydrogen production. The previously found optimal pH of 6.5 was held constant in all digesters, as well as the 12-hour HRT. Organic loading rates of 12, 18, 24, and 30 g COD/L-day were tested over two experiments.

OLR Experiment 1 examined COD loadings of 18, 24, and 30 g COD/L-day.

Digester feedstocks contained 7.4 g glycerol/L for digesters operating at an OLR of 18 g COD/L-day, 10 g glycerol/L for digesters operating at 24 g COD/L-day, and 12.33 g glycerol/L for digesters operating at an OLR of 30 g COD/L-day. D1 and D2 operated at an average OLR of 18.8 ± 0.1843 g COD/L-day (mean $\pm$ SD). D3 and D4 achieved an average OLR was 24.0 ± 0.8474 g COD/L-day, and

D5 and D6 had an average OLR of 28.8 ± 1.307 g COD/L-day. The average pH and alkalinity in all digesters was 6.35 and 3500 mg CaCO_3 /L respectively. Steady state conditions were identified for Days 10-14 for D1 and D2, and Days 12-16 for D3-6.

OLR Experiment 2 used glycerol as a substrate and tested organic loadings of 12, 18, and 24 g COD/L-day. Digester feedstocks contained 4.9 g glycerol for digesters operating at an OLR of 12 g COD/L-day, 7.4 g glycerol/L for digesters operating at 18 g COD/L-day, and 10 g glycerol/L for digesters operating at 24 g COD/L-day. D1 and D2 attained an average OLR of 12.7 ± 0.7986 g COD/L-day (mean $\pm$ SD). D3 and D4 achieved an average OLR of 17.6 ± 0.3489 g COD/L-day, and D5 and D6 had an average OLR of 23.1 ± 1.054 g COD/L-day. The average pH for all digesters was 6.52, while the average alkalinity was 4200 mg CaCO_3 /L. Steady state conditions were met for D1 and D2 on Day 7-11, for D3 and D4 on Day 10-14, and Day 11-15 for D5 and D6.

The final OLR experiment examined the use of food waste as a substrate. Previous research found that the optimal OLR for anaerobic digesters utilizing glucose as a substrate was 18 g glucose/L-day (19.2 g COD/L-day) (Olivas, 2015). At a 12-hr HRT, this loading corresponded to a concentration of 9 g glucose/L. Food waste glucose concentrations were estimated by volatile solids content (see methods 2-6) so food waste was diluted to achieve a concentration of 9 g glucose/L, or an organic loading rate of 18 g glucose/L-day. OLRs of 12 g glucose/L-day and 24 g glucose/L-day were also tested (Table 5).

Table 5. Target OLRs for food waste in concentration units of glucose and COD

Organic Loading Rate	
g glucose/L-day	g COD/L-day
12	12.8
18	19.2
24	25.6

D1 and D2 were fed concentrations of food waste targeting an OLR of 12.8 g COD/L-day; however, the mean OLR was 25.8 ± 3.97 g COD/L-day. D3 and D4 target OLR was 19.2 g COD/L-day, and a mean OLR of 31.2 ± 4.231 g COD/L-day was attained. D5 and D6 target OLR was 24 g COD/L-day, and a mean OLR of 33.9 ± 7.96 g COD/L-day was attained. The average pH and alkalinity of the digesters was 6.51 and 4700 mg CaCO₃/L. D1 and D2 reached steady state on Day 4-8, while D3-6 experienced steady state conditions on Day 31-35. D1 and D2 experienced leaks that were unable to be fixed, despite shutting down D1 and D2 for repair and restarting them on Day 14.

3.2.1 COD Balance

During the steady state period of OLR Experiment 1, digesters running at an OLR of 18.8 g COD/L-day recovered 92% of the influent COD, while digesters running at 24.0 g COD/L-day achieved 98% recovery of the influent COD feed. At an OLR of 28.8 g COD/L-day, gaseous and effluent COD was slightly greater than the influent COD, recovering 101% of influent COD (Figure 13).

Figure 13. COD balance for digesters operated at mean OLRs of 18.8, 24.0, and 28.8 g COD/L-day. OLR values were calculated as the mean over the course of the steady state period. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors ($n = 2$) shown on the digester effluent and biogas bars. The influent standard errors were based on measurements during the steady state performance period ($n = 5$).

During the steady state period of OLR Experiment 2, OLRs of 12.7 and 17.6 g COD/L-day recovery was 96% of influent COD (Figure 14). At an OLR of 23.1 g COD/L-day, 91% of influent COD was recovered.

Figure 14. COD balance for digesters operated at mean OLRs of 12.7, 17.6, and 23.1 g COD/L-day. OLR values were calculated as the mean over the course of the steady state period. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors ($n = 2$) shown on the digester effluent and biogas bars. The influent standard errors were based on measurements during the steady state performance period ($n = 5$).

Food waste-fed digesters operating at an OLR of 25.8 g COD/L-day with food waste had a recovery of 87% of the influent COD (Figure 15). At organic loading rates of 31.2 g COD/L-day and 33.9 g COD/L-day, the recoveries were 90% and 95%, respectively.

Figure 15. COD balance for food waste-fed digesters operated at mean OLRs of 25.8, 31.2, and 33.9 g COD/L-day. OLR values were calculated as the mean over the course of the steady state period. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors ($n = 2$) shown on the digester effluent and biogas bars. The influent standard errors were based on measurements during the steady state performance period ($n = 5$).

3.2.2 Molar Hydrogen Yields and Volumetric Hydrogen Production

Molar hydrogen yields observed at 18 and 24 g COD/L-day were less than 10% different between replicate experiments, indicating reproducible results. Molar hydrogen production was highest at a mean OLR of 18.2 g COD/L-day, producing 0.015 ± 0.0021 mol H_2 /mol COD (mean $\pm$ SE). A yield of 0.010 ± 0.0011 mol H_2 /mol COD was produced at an OLR of 12.7 g COD/L-day, and a yield of 0.008 ± 0.0002 mol H_2 /mol COD was produced at a mean OLR of 23.6 g COD/L-day. The highest OLR, at 28.8 g COD/L-day, produced the lowest molar yield of 0.005 ± 0.0002 mol H_2 /mol COD (Figure 16).

Figure 16. Molar hydrogen yields for OLRs of 12.7, 18.2, 23.6, and 28.8 g COD/L-day. OLRs of 18.2 and 23.6 g COD/L-day were calculated mean OLRs between replicate experiments. Standard error bars for those OLRs represent the error between replicate experiments (n=2). OLRs of 12.7 and 28.8 were tested once, so standard error bars represent the error among duplicate digesters (n=2).

Volumetric hydrogen production for glycerol-fed digesters was highest at an OLR of 18.2 g COD/L-day and produced 0.199 ± 0.0218 LH₂/L_{Reactor}-day (mean $\pm$ SE). Digesters operating at an OLR of 23.55 g COD/L-day produced the second highest volumetric hydrogen yield of 0.148 ± 0.0003 LH₂/L_{Reactor}-day. At 28.8 g COD/L-day 0.114 ± 0.0034 LH₂/L_{Reactor}-day was produced. The lowest volumetric hydrogen production was observed at an OLR of 12.7 g COD/L-day and was 0.091 ± 0.0102 LH₂/L_{Reactor}-day (Figure 17). Both molar hydrogen yields and volumetric hydrogen production for glycerol-fed digesters were highest at an OLR of 18.2 g COD/L-day.

Figure 17. Volumetric hydrogen yields for OLRs of 12.7, 18.2, 23.6, and 28.8 g COD/L-day. OLRs of 18.2 and 23.6 g COD/L-day were calculated mean OLRs between replicate experiments. Standard error bars for those OLRs represent the error between replicate experiments (n=2). OLRs of 12.7 and 28.8 were tested once, so standard error bars represent the error among duplicate digesters (n=2).

The highest molar hydrogen yield for food waste-fed digesters occurred at an OLR of 33.9 g COD/L-day and was 0.007 ± 0.0016 mol H₂/mol COD (mean $\pm$ SE) (Figure 18). A molar hydrogen yield of 0.005 ± 0.0016 mol H₂/mol COD was produced at the second highest OLR of 31.2 g COD/L-day. The Lowest OLR, 25.8 g COD/L-day, produced 0.004 ± 0.0012 mol H₂/mol COD.

Figure 18. Molar hydrogen yields for food waste-fed digesters at OLRs of 25.8, 31.2, and 33.9 g COD/L-day. OLR values represent the average OLR over the steady state period. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors shown (n = 2).

The volumetric hydrogen production for food waste-fed digesters was highest at highest OLR of 33.9 g COD/L-day and was $0.177 \pm 0.0373 \text{ LH}_2/\text{L}_{\text{Reactor-day}}$ (mean $\pm$ SE). At an OLR of 31.2 g COD/L-day, $0.117 \pm 0.0314 \text{ LH}_2/\text{L}_{\text{Reactor-day}}$ was produced. The lowest volumetric hydrogen yield occurred at an OLR of 25.8 g COD/L-day and was $0.080 \pm 0.0255 \text{ LH}_2/\text{L}_{\text{Reactor-day}}$ (Figure 19). Among all OLRs tested for food waste, the highest OLR, 33.9 g COD/L-day, produced the most hydrogen. It is likely that volumetric hydrogen production would increase at OLRs higher than 33.9 g COD/L-day.

Figure 19. Volumetric hydrogen production for food waste-fed digesters at OLRs of 25.8, 31.2, and 33.9 g COD/L-day. OLR values represent the average OLR over the steady state period. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors shown (n = 2).

3.3 HRT Experiments

Three HRT experiments were performed to test the effect of variable residence times on hydrogen production. The previously found optimal pH of 6.5 and OLR of 18 g COD/L-day were held constant amongst all glycerol-fed digesters. HRTs of 3, 6, 9, 12, and 18 hours were tested.

HRT Experiment 1 tested HRTs of 6, 12, and 18 hours. The average pH was 6.53 amongst all digesters, while the average alkalinity was 3700 mg CaCO₃/L. The mean OLR was 19.7 ± 1.88 g COD/L-day and was within one standard deviation of the target optimal OLR of 18 g COD/L-day (mean $\pm$ SD). Steady state conditions were met on Day 6-10 for D1-2, Day 7-11 for D3-4, and Day 15-19 for D5-6. Steady-state periods varied for each set of duplicate digesters due

to the steady-state criteria that said that at least 4 HRTs are to have passed before digesters were considered in steady state.

HRT Experiment 2 examined the HRTs of 3, 6, and 9 hours. The mean pH amongst the digesters was 6.58, while the mean alkalinity was 3800 mg CaCO_3/L . The mean OLR was 19.6 ± 1.67 g COD/L-day and was within one standard deviation of the target OLR of 18 g COD/L-day (mean $\pm$ SD). Steady state periods were met for D1-2 on Day 3-7, Day 4-8 for D3-4, and Days 8-12 for D5-6.

HRT Experiment 3 was a repeat of HRT Experiment 1 and examined HRTs of 6, 12, and 18 hours. Experiment 3 was repeated to ensure the data was repeatable. The mean pH was 6.44, while the mean alkalinity was 3600 mg CaCO_3/L . The OLR was within one standard deviation of the target OLR of 18 g COD/L-day and was 19.5 ± 1.75 g COD/L-day (mean $\pm$ SD).

3.3.1 COD Balance

HRT Experiment 1 achieved 104%, 81%, and 94% recovery for digesters operating at HRTs of 6, 12, and 18 hours (Figure 20). At a 6-hour HRT, slightly more COD was recovered in the effluent and gaseous COD than was fed. Influent COD measurements were inaccurate. The effluent and gaseous COD standard error was contained within the error bounds of influent COD, adding to the credibility of the hydrogen yields. The low recovery observed at a 12-hour HRT was a result of a leaking digester that was noted during the experiment

Figure 20. COD balance for HRT Experiment 1 with digesters operated at HRTs of 6, 12, and 18 hours. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors ($n = 2$) shown on the digester effluent and biogas bars. The influent standard errors were based on measurements during the steady state performance period ($n = 5$).

HRT Experiment 2 recovered 97%, 79%, and 81% of influent feed COD (Figure 21). Hydrogen yields produced by digesters operating at a 3-hour HRT were accepted due to the high recovery; however, leaking digesters were noted at the 6- and 9-hour HRTs, confirmed by the lower COD recoveries and indicating lower confidence in their hydrogen yield values.

Figure 21. COD balance for digesters operated at HRTs of 3, 6, and 9 hours. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors ($n = 2$) shown on the digester effluent and biogas bars. The influent standard errors were based on measurements during the steady state performance period ($n = 5$).

Recoveries of 97%, 93%, and 86% were achieved for digesters in the HRT Experiment 3 operating at HRTs of 6, 12, and 18 hours (Figure 22).

Figure 22. COD balance for digesters operated at HRTs of 6, 12, and 18 hours. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors ($n = 2$) shown on the digester effluent and biogas bars. The influent standard errors were based on measurements during the steady state performance period ($n = 5$).

3.3.2 Molar Hydrogen Yields and Volumetric Hydrogen Production

Among the three HRT experiments run, HRTs of 6, 12, and 18 hours were repeated. HRTs of 3 and 9 hours were tested once and represent the average molar hydrogen yield and volumetric hydrogen production between digester duplicates.

The highest molar yield was observed at HRTs of 6 and 12 hours. At an HRT of 6 hours, a molar hydrogen yield of 0.010 ± 0.0032 mol H_2 /mol COD was observed, while at an HRT of 12 hours, a molar yield of 0.010 ± 0.0000 mol H_2 /mol COD was observed (mean $\pm$ SE) (Figure 23). A molar yield of 0.009 ± 0.0004 mol H_2 /mol COD was obtained at an HRT of 12 hours, and 0.008 ± 0.0014 mol

H₂/mol COD at 18 hours. The lowest molar hydrogen yield was observed at the shortest HRT of 3 hours and was 0.002 ± 0.0005 mol H₂/mol COD.

Figure 23. Molar yields for HRTs of 3, 6, 9, 12, and 18 hours. Standard error bars for HRTs of 6, 12, and 18 hours represent the standard error between replicate experiments (n=2). Standard error bars for 3- and 9-hour HRTs represent the error between duplicate digesters (n=2).

The volumetric hydrogen production was highest at an HRT of 12 hours and was 0.148 ± 0.0107 LH₂/L_{Reactor}-day (mean $\pm$ SE). A similar yield of 0.145 ± 0.0465 LH₂/L_{Reactor}-day was obtained for an HRT of 6 hours. At HRTs of 9 and 18 hours, volumetric hydrogen production of 0.127 ± 0.0041 LH₂/L_{Reactor}-day and 0.105 ± 0.0012 LH₂/L_{Reactor}-day were observed. The shortest HRT, 3 hours, produced the lowest volumetric hydrogen production of 0.027 ± 0.0072 LH₂/L_{Reactor}-day (Figure 24). Though HRTs of 6 and 12 hours produced similar molar hydrogen yields and volumetric hydrogen production, the standard error was considerably less at a 12 hour HRT. The lower standard error provided for a greater degree of confidence in an HRT of 12 hours, so it was used for subsequent experiments.

Figure 24. Volumetric hydrogen production for HRTs of 3, 6, 9, 12, and 18 hours. Standard error bars for HRTs of 6, 12, and 18 hours represent the standard error between replicate experiments ($n=2$). Standard error bars for 3- and 9-hour HRTs represent the error between duplicate digesters ($n=2$).

3.4 Sparging Experiments

Previous research found that sparging glucose-fed digesters with nitrogen gas nearly doubled the molar hydrogen yields and volumetric hydrogen production (Olivas, 2015). Sparging experiments were performed for both glycerol and food waste substrates at varying sparging rates to examine their effect on hydrogen production.

The glycerol sparging experiment was operated at a target pH of 6.51, OLR of 18.2 g COD/L-day, and an HRT of 12 hours—conditions found to be optimal for hydrogen production in previous experiments. D1-2 were sparged with high purity nitrogen gas, D3-4 were unsparged and used as a control, and D5-6 were sparged with biogas (70% CH₄, 30% CO₂) from four separate on-site anaerobic

digesters. The sparging rates were dependent on the gas production rate from these digesters. However, sparging rates were low enough to ensure they could be sparged at a constant rate. Sparged digesters were always operated at the same sparging rate, regardless of gas type; however, the sparging rates were occasionally changed to observe any changes in hydrogen production. Sparging rates tested were 1.2, 2, 2.5, 3, and 3.2 mL/min.

The glycerol-fed digesters operated at a mean pH and alkalinity of 6.46 and 3500 mg CaCO₃/L. The mean OLR during the experiment was 17.8 ± 1.324 g COD/L-day (mean $\pm$ SD) and was within one standard deviation of the target OLR of 18.2 g COD/L-day. Steady state conditions were achieved on Day 13-17 for D1-2 and D5-6, and Day 23-27 for D3-4. The steady state performance criteria were met much later for D3-4 because gas production was steadily increasing until Day 23.

The food waste sparging experiment was operated at the target OLR of 33.9 g COD/L-day. pH and HRT optimization experiments were not run for digesters fed food waste, so values from glycerol-fed digesters were used (pH 6.51, HRT 12 hours). Digesters 1-2 and 5-6 were sparged with high purity nitrogen gas, while D3-4 were unsparged and used as a control. Sparging rates tested were 0.5 L/hr for D1-2, and 1.0 L/hr for D5-6. The mean pH and alkalinity was 6.40 and 4600 mg CaCO₃/L. The mean OLR was 32.9 ± 3.4944 g COD/L-day (mean $\pm$ SD). All digesters maintained steady state conditions for Days 7-11 of the experiment.

3.4.1 COD Balance

Glycerol-fed digesters sparged with nitrogen gas achieved an influent COD recovery of 95%, while the unsparged digesters achieved an influent COD recovery of 96%. Biogas-sparged digesters achieved a 92% recovery. Recovery for biogas-sparged digesters was high because the digesters were being sparged with biogas consisting of 70% methane which contributes to the gaseous COD (Figure 25).

Figure 25. COD balance for sparged and unsparged digesters. Digesters were operated at a mean OLR of 17.8 g COD/L-day, pH of 6.46, and HRT of 12 hours. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors ($n = 2$) shown on the digester effluent and biogas bars. The influent standard errors were based on measurements during the steady state performance period ($n = 5$)

Food waste-fed digesters sparged at a rate of 0.5 L N₂/hr recovered 86% of the influent COD while digesters sparged at a rate of 1.0 L N₂/hr recovered 102% of influent COD feed. The standard error in effluent and gaseous COD between digester duplicates at 1.0 L N₂/hr was within the standard error of influent COD

measurements, rather the inaccurate recovery is attributed to inaccurate influent COD measurements. The unsparged digesters recovered 93% of the influent COD measurements. The unsparged digesters recovered 93% of the influent COD feed. These recoveries indicate confidence in the hydrogen yields because it shows that the digesters were not leaking, and mass was not produced (Figure 26).

Figure 26. COD balance for food waste-fed digesters that were sparged or unsparged. Digesters were operated at a mean OLR of 32.9 g COD/L-day, pH of 6.40, and HRT of 12 hours. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors ($n = 2$) shown on the digester effluent and biogas bars. The influent standard errors were based on measurements during the steady state performance period ($n = 5$)

3.4.2 Molar Hydrogen Yields and Volumetric Hydrogen Production

Molar hydrogen yields and volumetric hydrogen production were calculated for glycerol-fed digesters during their steady state period, and for each sparging rate tested (Figure 27). Nitrogen and biogas sparged digesters, sparged at a rate of 3.2 mL/min, produced nearly the same molar yield of hydrogen, with nitrogen-sparged digesters producing 0.020 ± 0.0005 mol H₂/mol COD, and biogas-

sparged digesters producing 0.020 ± 0.0029 mol H_2 /mol COD (mean $\pm$ SE). The unsparged digesters produced 0.012 ± 0.0016 mol H_2 /mol COD. At steady state conditions, digesters that were sparged converted 40% more of the COD fed to them into hydrogen gas than the unsparged digesters. Similar molar hydrogen yields and volumetric hydrogen production for nitrogen gas and biogas was a surprising result.

Figure 27. Molar yields for unsparged (US), nitrogen sparged (NG), and biogas sparged (BG) digesters during the steady state period. Digesters operated at a pH of 6.46, HRT of 12 hours, and an OLR of 17.8 g COD/L-day. The sparging rate for NG and BG was 3.2 mL/min. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors shown ($n = 2$).

Volumetric hydrogen production for the glycerol-fed digesters at steady state were highest when sparged with biogas and nitrogen gas. Sparging rates for the steady state period were 3.2 mL/min. Biogas-sparged digesters produced 0.281 ± 0.0395 $LH_2/L_{Reactor}$ -day, while nitrogen-sparged digesters produced 0.269 ± 0.0091 $LH_2/L_{Reactor}$ -day (mean $\pm$ SE). Unsparged digesters produced $0.156 \pm$

0.0210 L H₂/L_{Reactor}-day. On average, sparged digesters produced 44% more hydrogen gas than the unsparged digesters (Figure 28).

Figure 28. Volumetric hydrogen production for unsparged (US), nitrogen sparged (NG), and biogas sparged (BG) digesters during the steady state period. The sparging rate for NG and BG was 3.2 mL/min. Digesters operated at a pH of 6.46, HRT of 12 hours, and an OLR of 17.8 g COD/L-day. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors shown (n = 2).

Molar hydrogen yields for glycerol-fed digesters were calculated at each sparging rate and compared to the unsparged digester at steady state (Figure 29). The highest molar yield occurred when both nitrogen and biogas-sparged digesters were sparged at a rate of 3.2 mL/min. At this sparging rate, nitrogen-sparged digesters produced 0.020 ± 0.0005 mol H₂/mol COD (mean $\pm$ SE), and biogas-sparged digesters produced 0.020 ± 0.0029 mol H₂/mol COD. The conversion of COD introduced to hydrogen gas increased with increasing sparging until reaching a sparging rate of 2.5 mL/min. At this sparging rate the molar yields began to stabilize, despite the increasing sparging rates.

Figure 29. Molar hydrogen yields for unsparged (US), nitrogen sparged (NG), and biogas sparged (BG) digesters at various sparging rates. Digesters operated at a pH of 6.46, HRT of 12 hours, and an OLR of 17.8 g COD/L-day. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors shown ($n = 2$). The leftmost bar in each set of sparging rates represents the steady state molar hydrogen yield of an unsparged digester running under the same conditions.

Figures 30 and 31 depict the mean molar hydrogen yields at each flow rate for the sparging gasses used. Molar hydrogen yield data for both nitrogen and biogas sparging were consistent with a linear fit.

Figure 30. Molar hydrogen yields for digesters sparged with nitrogen gas (NG). The linear fit produced an R^2 value of 0.93, and error bars depict the standard error produced between duplicate digesters ($n=2$).

Figure 31. Molar hydrogen yields for digesters sparged with biogas (BG). The linear fit produced an R^2 value of 0.95, and error bars depict the standard error produced between duplicate digesters ($n=2$).

Volumetric hydrogen production was highest at the flow rate of 3.2 mL/min.

Volumetric hydrogen production of 0.281 ± 0.0395 $\text{LH}_2/\text{L}_{\text{Reactor}}\text{-day}$ were observed for biogas-sparged digesters, and 0.269 ± 0.0091 $\text{LH}_2/\text{L}_{\text{Reactor}}\text{-day}$ was produced by nitrogen-sparged digesters (mean $\pm$ SE). Hydrogen production increased with increasing sparging rates for both nitrogen and biogas-sparged digesters. Unlike the trend seen for molar yields, the volumetric hydrogen production does not stabilize above the sparging rate of 2.5 mL/min (Figure 32). Yields may increase at sparging rates higher than 3.2 mL/min.

Figure 32. Volumetric hydrogen production for unsparged (US), nitrogen sparged (NG), and biogas sparged (BG) digesters at varying sparging rates. Digesters operated at a pH of 6.46, HRT of 12 hours, and an OLR of 17.8 g COD/L-day. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors shown ($n = 2$). The leftmost bar in each set of sparging rates represents the steady state volumetric hydrogen production of an unsparged digester running under the same conditions.

Figures 33 and 34 depict the mean volumetric hydrogen production at each flow rate and gas tested. Volumetric hydrogen production data for both nitrogen and

biogas sparging were consistent with a linear fit, resulting in R^2 values of 0.94 and 0.96 respectively.

Figure 33. Volumetric hydrogen production for digesters sparged with nitrogen gas (NG). The linear fit produced an R^2 value of 0.94, and the means of steady-state performance periods by duplicate digesters were used to calculate the standard errors shown ($n = 2$).

Figure 34. Volumetric hydrogen production for digesters sparged with biogas (BG). The linear fit produced an R^2 value of 0.95, and the means of steady-state performance periods by duplicate digesters were used to calculate the standard errors shown ($n = 2$).

Molar yields for food waste-fed nitrogen-sparged digesters were highest for digesters sparged at 1 L N₂/hr. At this sparging rate, the molar hydrogen yield was 0.021 ± 0.0013 mol H₂/mol COD (mean $\pm$ SE) (Figure 35). At the lowest sparging rate tested, 0.5 L N₂/hr, a molar yield of 0.014 ± 0.0066 mol H₂/mol COD was observed. The unsparged digester produced 0.005 ± 0.0006 mol H₂/mol COD. The unsparged molar yield was low compared to the previously measure molar yield of 0.007 ± 0.0016 mol H₂/mol COD for food waste-fed digesters running under the same conditions. By sparging the digesters with 1 L N₂/hr, 76% more of the influent COD was converted into hydrogen gas than the unsparged digester. A sparging rate of 0.5 L N₂/hr produced 64% more hydrogen per mol of COD than the unsparged digester. Molar hydrogen yields increased linearly with sparging rate, resulting in an R^2 value of 0.99 (Figure 36).

Figure 35. Molar hydrogen yields for food waste-fed digesters that were either unsparged (US) or sparged at flowrates of 0.5 and 1 L N₂/hr. All digesters operated at a mean OLR of 32.9 g COD/L-day, pH of 6.40, and HRT of 12 hours. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors shown (n=2).

Figure 36. Molar hydrogen yields for food waste-fed digesters sparged with nitrogen gas. The linear fit produced an R^2 value of 0.99, and the means of steady-state performance periods by duplicate digesters were used to calculate the standard errors shown (n = 2).

Volumetric hydrogen production for food waste-fed and nitrogen-sparged digesters were the highest of all substrates and conditions tested. Digesters sparged with 1 L N₂/hr produced a volumetric hydrogen production of 0.478 ± 0.0280 L H₂/L_{Reactor}-day (mean $\pm$ SE). At the lowest sparging rate tested, 0.5 L N₂/hr, a volumetric hydrogen production of 0.382 ± 0.1049 L H₂/L_{Reactor}-day was observed. The unsparged digester produced 0.123 ± 0.0139 L H₂/L_{Reactor}-day (Figure 37). By sparging the digesters with 1 L N₂/hr, 74% more hydrogen was produced than the unsparged digester. A sparging rate of 0.5 L N₂/hr produced 64% more hydrogen per mol of COD than the unsparged digester. Volumetric hydrogen production increased linearly with sparging rate, resulting in an R² value of 0.93 (Figure 38).

Figure 37. Volumetric hydrogen production for nitrogen-sparged and food waste-fed digesters operating at a mean OLR of 32.9 g COD/L-day, pH of 6.40, and HRT of 12 hours. The means of steady-state performance periods by duplicate digesters were used to calculate the standard errors shown (n = 2).

Figure 38. Volumetric hydrogen production for food waste-fed digesters sparged with nitrogen gas. The linear fit produced an R^2 value of 0.93, and the means of steady-state performance periods by duplicate digesters were used to calculate the standard errors shown ($n = 2$).

CHAPTER 4: DISCUSSION

Results from each of the hydrogen optimization experiments show that pH, OLR, HRT, gas sparging, and substrate (glycerol or food waste) each individually affected hydrogen yields and production. Optimal conditions were defined as those producing the highest molar hydrogen yield or volumetric hydrogen production. The optimal operational conditions for glycerol substrate were a pH of 6.51, OLR of 18.8 g COD/L-day, HRT of 12 hours, and biogas or nitrogen sparging rate of 3.2 mL/min (0.2 L/hr) Glycerol-fed digesters operating at these variable levels produced a molar yield of 0.020 ± 0.0029 mol H₂/mol COD and a volumetric hydrogen production of 0.281 ± 0.0395 LH₂/L_{Reactor}-day.

Three-dimensional surface plots of HRT, OLR, and hydrogen yield and production data were produced to graphically represent the data obtained in experiments fed glucose substrate. The surface plots depict the ways in which HRT and OLR affect hydrogen yield and production for unsparged digesters (Figures 39 & 40). These optimal conditions were determined before the sparging experiments to assess the effect of sparging on hydrogen production. The peak of each chart represents the highest molar hydrogen yield or volumetric hydrogen production. As values of HRT and OLR deviate from the conditions producing the peak (OLR 18.2 g COD/L-day, HRT 12 hours) yields begin to decrease.

Figure 39. Molar hydrogen yields as a function of HRT and OLR within a pH range of 6.35 to 6.58. The optimal molar hydrogen yield is the peak of the surface plot (0.012 mol H₂/mol COD) at an OLR value of 18.2 g COD/L-day and HRT of 12 hours.

Figure 40. Volumetric hydrogen production as a function of HRT and OLR within a pH range of 6.35 to 6.58. The optimal volumetric hydrogen production is the peak of the surface plot ($0.156 \text{ L H}_2/\text{L}_{\text{Reactor}}\text{-day}$) at an OLR value of 18.2 g COD/L-day and HRT of 12 hours.

Hydrogen optimization experiments for food waste substrate only focused on optimizing OLR and sparging rates because the optimal conditions for pH and HRT determined in glycerol experiments were used. Food waste-fed digesters operating at an OLR of 32.9 g COD/L-day , pH of 6.51, HRT of 12 hours, and sparging rate of $1 \text{ L N}_2/\text{hr}$ produced a molar hydrogen yield of $0.021 \pm 0.0013 \text{ mol H}_2/\text{mol COD}$ and volumetric hydrogen production of $0.478 \pm 0.0280 \text{ L H}_2/\text{L}_{\text{Reactor}}\text{-day}$.

The presence of methane indicates the presence of methanogenic bacteria which can consume hydrogen, potentially reducing hydrogen yields (Gunaseelan, 1997; Thompson, 2008). Based on gas chromatography that reported no methane gas, methanogenic bacteria did not have an effect on hydrogen yields

for any of the hydrogen optimization experiments. Methane gas was not observed over the steady state periods in which hydrogen yields were calculated, except for the biogas (70% methane, 30% carbon dioxide) sparged digesters. Methane gas was not produced by the biogas sparged digesters, it was only present because the sparging gas, biogas, contained methane at the start of each experiment, methane production was observable prior to the complete washout of the methanogens in the inoculum; typically three residence times.

4.1 Comparisons to Literature

Results, specifically molar hydrogen yields, are compared to results from previous studies to understand the way in which reactor types, microorganisms, and operational conditions impact fermentative hydrogen production (Table 7). Molar yields were calculated on a COD-fed per day basis to allow comparisons among various substrates, including undefined ones such as food waste. The oxygen demand for one mole of glycerol, for example, is 3.5 moles of oxygen (Equation 4-1). Molar hydrogen yields were converted COD introduced to glycerol introduced (Equation 4-2, Table 6).

$$\frac{0.013 \text{ mol } H_2}{\text{mol COD}} \times \frac{3.5 \text{ mol COD}}{\text{mol glycerol}} = \frac{0.047 \text{ mol } H_2}{\text{mol glycerol}} \quad (\text{Eq. 4-2})$$

Table 6. Molar yields observed at the optimal operational conditions with respect to COD and glycerol

pH	OLR (g COD/L-day)	HRT (hours)	Sparging Rate (mL/min)	Molar Hydrogen Yield	
				mol H ₂ / mol COD	mol H ₂ / mol glycerol
6.51	23.8 ± 1.502	12	0	0.013 ± 0.0029	0.047 ± 0.0102
6.40	18.2 ± 0.6353	12	0	0.015 ± 0.0021	0.051 ± 0.0074
6.48	19.7 ± 1.493	12	0	0.010 ± 0.0000	0.035 ± 0.0002
6.39	17.7 ± 0.9010	12	0	0.012 ± 0.0016	0.041 ± 0.0055
6.49	18.7 ± 2.046	12	3.2	0.020 ± 0.0029	0.071 ± 0.0100

The anaerobic digestion of glycerol yields numerous products including hydrogen, carbon dioxide propionic acid, succinic acid, butanol and ethanol (Yazdani & Gonzalez, 2007). These products are formed through several fermentation pathways and are dependent on the environmental conditions and the type of microorganism involved in the fermentation process (Dharmadi, Muraka, & Gonzales, 2006; Gonzales, Pelayo-Ortiz, Bories, Jauregui, & Himmi, 2004; Yazdani & Gonzalez, 2007). One potential fermentation pathway converts glycerol to ethanol, hydrogen, and carbon dioxide (Equation 4-3). Following this fermentation pathway, by stoichiometry and assuming no heat losses, one mole of hydrogen can theoretically be produced from one mole of glycerol. Biological yields are frequently significantly less than stoichiometric yields, possibly down to half (Hallenbeck & Benemann, 2002).

One factor affecting fermentation pathways is the type of microorganism, or culture used to ferment the substrate. Many experiments have performed

fermentative hydrogen production with pure cultures known to ferment glycerol to hydrogen. One study operating at an OLR of 24.3 g COD/L-day and an HRT of 12 hours produced 0.38 mol H₂/mol glycerol when inoculated with *Clostridium butyricum* LMG 1212t2 (Heyndrickx, Vos, & Vancanneyt, 1991). This molar hydrogen yield is greater than what was found in this experiment. However, the use of pure cultures on a larger scale is not plausible because preventing contamination is challenging, especially with substrates that may already contain microorganisms (i.e. food waste). Energy intensive sterilization, or inactivation of microorganisms contained in the substrate may be required, and even then, contamination is still possible (Masset et. al. 2012).

The use of mixed cultures is of greater benefit to large scale systems because fluctuations in microbial communities have little impact on overall hydrogen production (Masset et al., 2012; Agler, Wrenn, Zinder, & Angenent, 2011). The downfall to mixed cultures is that hydrogen yields are generally much lower than pure cultures (Masset et al., 2012). Anaerobic sludge used as an inoculum for a continuously stirred tank reactor produced 0.04 mol H₂/mol glycerol at pH of 6.5, OLR of 24.3 g COD/L-day and an HRT of 12 hours (Silva-Illanes et al., 2015). This yield, was ten times lower than the observed yield of 0.38 mol H₂/mol glycerol obtained by Heyndrickx et al., whose reactors were run at the same OLR and HRT as Silva-Illanes et al., but were inoculated with a pure culture (1991, 2015). A nearly identical molar hydrogen yield to that found by Silva-Illanes et al. was observed in this experiment at the same OLR and HRT. Digesters in this experiment produced 0.047 ± 0.0102 mol H₂/mol glycerol and were inoculated

with a mixed culture, anaerobic sludge, and run at a pH of 6.5, OLR of 23.8 g COD/L-day and HRT of 12 hours.

Table 7. Molar hydrogen yields obtained from various experiments using pure glycerol as a substrate. Each yield was generally higher than what was observed in this experiment.

pH	Reactor	Organic Loading Rate (g COD/L-day)	HRT (hours)	Yield (mol H ₂ /mol glycerol)	Culture	Source
5.5	CSTR	24.3	12	0.41	Anaerobic Sludge	Silva-Illanes et al. (2015)
6.5	CSTR	24.3	12	0.04	Anaerobic Sludge	Silva-Illanes et al. (2015)
6.5	CSTR	36.5	8	0.17	Anaerobic Sludge	Silva-Illanes et al. (2015)
5.5	CSTR	12.2	12	0.40	Anaerobic Sludge	Tapia-Venegas et al. (2015)
-	Batch	24.3 (g COD/L)	-	0.53	<i>Klebsiella pneumoniae</i>	Liu and Fang (2007)
-	CSTR	24.3	12	0.38	<i>Clostridium butyricum</i> LMG 1212t2	Heyndrickx et al. (1991)
-	CSTR	24.3	12	1.05	<i>Clostridium pasteurianum</i> LMG 3285	Heyndrickx et al. (1991)
-	CSTR	24.3	12	0.50	<i>Clostridium pasteurianum</i> CH4	Lo et al. (2013)
-	Batch	12.1 (g COD/L)	-	0.41	Anaerobic Sludge	Seifert et. al. (2009)

pH was found to have a great impact on hydrogen production by Silva-Illanes et al., who observed the highest molar yield of 0.41 mol H₂/mol glycerol was obtained at a pH of 5.5. Similarly, a molar yield of 0.40 mol H₂/mol glycerol was achieved at the same pH in a separate study (2015). The major difference between these two studies was that Silva-Illanes et al. operated at an OLR of 24.3 g COD/L-day, while Tapia-Venegas et al. operated at an OLR of 12.2 g COD/L-day (2015). Even though the OLRs between the two experiments were nearly 50% different, a similar molar hydrogen yield was achieved. The molar

yield observed by Silva-Illanes et al. at a pH of 6.5 was ten times smaller than what was discovered at a pH of 5.5 (2015). At the lowest pH tested in this experiment, 6.15, molar hydrogen yields only reached 0.02 mol H₂/mol glycerol, despite being run at a similar OLR and HRT to Silva-Illanes et al. (2015). It is possible that the yields found in this experiment at a pH of 6.15 were lower than what Silva-Illanes et al. and Tapia-Venegas et al. observed when operating at a pH of 5.5 because of the difference in pH may have selected for microorganisms in the anaerobic sludge that better fermented glycerol.

In addition to fermentation pathways and environmental conditions, the type of reactor used for fermentative hydrogen production also seems to influence molar hydrogen yields and production. Liu and Fang observed a molar hydrogen yield of 0.53 mol H₂/mol glycerol with an anaerobic sludge inoculum in a CSTR at an organic loading of 24.3 g COD/L-day (2007). Seifert et al. observed a molar hydrogen yield of 0.41 mol H₂/mol glycerol at an organic loading of 12.1 g COD/L (2009). Numerous studies utilize batch reactors to produce hydrogen from different organic wastes, yet like using pure cultures, batch reactors have some disadvantages that make their use less desirable (Batstone, Torrijos, Ruiz, Schmidt, 2004). To start, achieving similar results and products for each batch reaction may be difficult if mixed cultures are used as an inoculum. If the conditions inside the reactors are not the same as in the studies cited above. The time it takes to ferment the substrate in batch reactors is also longer than that of a continuous stirred tank reactor (Batstone et al., 2004). Continuous reactors are

more desirable for larger scale fermentative hydrogen production because continuous production of hydrogen from a steady flow of substrate is achieved.

High hydrogen partial pressures in the liquid phase was found to be one of the main factors affecting hydrogen production (Mizuno et al., 2000). One study found that the digestion of glycerol was inhibited by dissolved hydrogen gas in the liquid phase because the metabolism of the microorganism involved in the fermentation process was inhibited (Dharmadi et al., 2006). Stripping the gas out of the liquid phase would therefore decrease the hydrogen partial pressure and allow for a greater amount of glycerol to be digested and converted into products like hydrogen gas. To increase the yields observed in pH, OLR, and HRT experiments, gas sparging of the digesters was tested.

The sparging of digesters with high purity nitrogen gas and biogas was found to nearly double the molar hydrogen yield in glycerol-fed digesters. The study showed that hydrogen yields differed by only 1.4% when sparged with nitrogen or biogas, indicating that the type of sparging gas does not influence the resulting hydrogen yields and production. The unsparged digesters operating at a pH of 6.39, OLR of 17.7 ± 0.9010 g COD/L-day, and an HRT of 12 hours produced a molar hydrogen yield of 0.041 ± 0.0055 mol H₂/mol glycerol. Nitrogen and biogas sparged digesters produced a molar yield of 0.071 ± 0.0100 mol H₂/mol glycerol at an OLR of 18.7 ± 2.046 g COD/L-day, 12 hour HRT, and pH of 6.49.

Unsparged digesters produced only 4.1% of the theoretical hydrogen yield for glycerol, while sparged digesters produced 7.1% of the theoretical hydrogen

yield. A useful comparison to the sparged molar hydrogen yields for glycerol are those observed with glucose substrate because glucose is, theoretically, the best substrate for fermentative hydrogen production. The theoretical molar hydrogen yield for glucose is 4 mol H₂/mol glucose. Kim et al. studied the effect of variable sparging rates of biogas, and nitrogen on molar hydrogen yields. Digesters were operated at a pH of 5.3, OLR of 40 g COD/L-day, and an HRT of 12 hours. The unsparged, control digester obtained a molar yield of 0.75 mol H₂/mol glucose, while digesters sparged with biogas and nitrogen gas achieved molar hydrogen yields of 0.84, and 0.87 mol H₂/mol glucose respectively (Kim, Han, Kim, & Shin, 2006). Sparging the digesters caused a 12% increase in molar hydrogen yields, and attained about 22% of the theoretical molar hydrogen yield for glucose.

In a separate experiment, glucose-fed digesters operating within a pH range of 6.0-6.40, an HRT of 12 hours, and an OLR of 19.2 g COD/L-day produced a molar yield of 0.61 mol H₂/mol glucose. When sparged with 10.7 L N₂/hr, molar hydrogen yields increased to 3.08 mol H₂/mol glucose (Olivas, 2015). When sparged with 10.7 L N₂/hr, hydrogen yields increased by 500%, producing roughly 77% of theoretical molar hydrogen yield for glucose. The differences between the yields obtained by Olivas and Kim et al. are likely attributed to the different OLRs, pH values, as well as sparging rates.

Sparging digesters in this experiment increased the overall molar hydrogen yield by 42%, which is 7.1 % of the theoretical molar hydrogen yield for glycerol. However, the highest sparging rate tested was 3.2 mL/min per liter of digester,

far less than the sparging rate of 10.7 L N₂/hr tested by Olivas, and 6 L N₂/hr tested by Kim et al. Sparging rates tested for glycerol-fed digesters were plotted against the molar hydrogen yields produced at each sparging rate to produce Equation 4-4. As stated earlier, it is likely that only 50% (0.5 mol H₂/mol glycerol) of the theoretical molar hydrogen yield for glycerol is actually attainable for fermentative hydrogen production. Assuming the relationship remains linear, 0.5 mol H₂/mol glycerol was substituted for y in Equation 4-4, and resulted in a sparging rate of 44 mL/min. At this sparging rate, it is estimated that 0.5 mol H₂/mol glycerol could be produced.

$$y = 0.003x + 0.0106 \quad (\text{Eq. 4-4})$$

Food waste-fed and nitrogen sparged digesters produced molar hydrogen yields that increased, almost linearly, with an increasing sparging rate. A linear regression was performed on sparging rates of 0 L N₂/hr (unsparged), 0.5 L N₂/hr, and 1.0 L N₂/hr for molar hydrogen yields resulting in an R² of 0.99 (Equation 4-5). The highest molar hydrogen yield was accomplished at a sparging rate of 1.0 L N₂/hr and was 0.021 ± 0.0013 mol H₂/mol COD. Compared to glycerol, food waste achieved molar hydrogen yields that were 5% greater than the maximum molar hydrogen yield for glycerol.

$$y = 0.0158x + 0.0054 \quad (\text{Eq. 4-5})$$

When sparged with nitrogen gas at 1.0 L N₂/hr, the molar hydrogen yield obtained in this experiment (0.021 ± 0.0013 mol H₂/mol COD) was lower than what was found in other experiments (Table 8). It is important to note that pH and

HRT optimization experiments were not performed for the food waste substrate, so while molar hydrogen yields obtained in this experiment may be low, there is a chance that hydrogen yields would increase once the optimal pH and HRT are found. One experiment, operating at a pH of 6.0 and OLR of 28 g COD/L-day, obtained a molar hydrogen yield of 0.054 mol H₂/mol COD (Lee et al., 2010). The major differences between Lee's experiments were pH and inoculum. Lee et al. inoculated the reactors with enriched kitchen waste compost and maintained a pH of 6.0. It is possible that the microorganisms in the kitchen waste compost may have been better adapted to food waste fermentation. A separate experiment operated at an OLR of 50 g COD/L-day, HRT of 2 days, and pH of 5.5, produced a molar hydrogen yield of 0.038 mol H₂/mol COD (Li et al., 2008). The food waste sparging experiments likely produced lower hydrogen yields than those obtained by Li et al. because of the differences in HRT, OLR, and pH.

Table 8. Molar hydrogen yields produced by food waste substrate at varying operational conditions and cultures.

pH	Reactor	Organic Loading Rate (g COD/L-day)	HRT (day)	Yield (mol H ₂ /mol COD)	Culture	Source
-	Batch	4.6	-	0.135	Anaerobic sludge	Chen et al. (2006)
5.5	CSTR	50	2	0.038	Acidogenic sludge	Li et al. (2008b)
6.0	CSTR	28	-	0.054	Kitchen Waste Compost	Lee et al. (2010a)

Molar hydrogen yields obtained in this experiment were, for the most part, lower than yields obtained in experiments (Table 7). Culture-type and pH are likely the reasons yields were so low. As stated earlier, glycerol can be fermented to

various products: *Escherichia coli*, for example, can ferment glycerol to hydrogen gas, but only under acidic conditions and when hydrogen is not accumulating in the liquid phase (Yazdani & Gonzalez, 2007; Dharmadi et al., 2006). On the other hand, the microorganism *Propionibacteria acidipropioncic* will ferment glycerol to propionic acid (Yazdani & Gonzalez, 2007; Gonzales et al., 2004). It is unknown whether either of these microorganisms were present in the initial anaerobic sludge inoculum. However, experiments that used anaerobic wastewater sludge still produced higher molar yields. The major difference was pH. Experiments yours or others? operating at the same OLR and HRT produced higher molar hydrogen yields when operating at a pH of 5.5 than were produced at a pH of 6.5. However, molar hydrogen yields produced in this experiment were highest at a pH of 6.5. Because molar hydrogen yields at 6.5 were consistently better than those obtained at a pH of 6.15, pH values less than 6.15 were not tested. Future experiments using mixed cultures as a substrate should focus on testing a greater range of pH values to ensure the optimal pH is found.

Food waste-fed digesters also produced molar hydrogen yields that were lower than those found in other experiments. Unlike glycerol experiments, pH and HRT optimization experiments were not run for food waste substrate, rather the optimal pH and HRT from glycerol experiments were used. Therefore, food waste experiments were not run at the optimal pH and HRT. It is likely that the molar hydrogen yields for food waste would have been higher had pH and HRT optimization experiments been run. Future HRT and pH testing is necessary to determine all of the operational conditions for food waste.

CHAPTER 5: CONCLUSION

A series of experiments were performed to individually optimize each of the major operational conditions affecting fermentative hydrogen production. The major operational conditions tested were pH, OLR, HRT, and gas sparging rate. A range of values for each condition were tested, and those that produced the highest molar hydrogen yield and volumetric hydrogen production were deemed optimal and used in subsequent experiments. It was determined that the optimal pH, OLR, HRT, and gas sparging rate for glycerol substrate were pH 6.5, 18.2 g COD/L-day, 12 hours, and 3.2 mL/min per liter of digester. At these conditions, a molar hydrogen yield of 0.020 ± 0.0029 mol H₂/mol COD and volumetric hydrogen production of 0.281 ± 0.0395 L H₂/L_{Reactor}-day were produced. Molar hydrogen yields obtained in this experiment were low compared to other experiments.

Separate experiments performed for food waste substrate established an optimal OLR of 33.9 g COD/L-day and nitrogen sparging rate of 1.0 L N₂/hr. pH and HRT optimization experiments were not performed for food waste substrate, though it is recommended they be performed in future experiments. Digesters fed food waste substrate produced a maximum molar hydrogen yield of 0.021 ± 0.0013 mol H₂/mol COD and volumetric hydrogen production of 0.478 ± 0.0280 L H₂/L_{Reactor}-day at a pH of 6.5 and HRT of 12 hours.

Gas sparging of anaerobic digesters, a novel approach to increasing overall hydrogen yields and production, was examined for glycerol and food waste

substrate. For all sparging rates examined, molar hydrogen yields and volumetric hydrogen production increased almost linearly with sparging rate. Relationships between gas sparging rate and molar hydrogen yields were produced for each of the substrates tested, resulting in coefficients of determination, or R^2 values, greater than 0.92. Assuming the relationships remain linear, future experiments can estimate, and examine the potential increase in molar hydrogen yields at increased sparging rates. Compared to unsparged digester, sparged digesters increased molar hydrogen yields and volumetric hydrogen production by at least 42%. Most importantly, it was determined that the type of gas involved in sparging had very little, if any, effect on overall hydrogen production.

After performing numerous experiments on hydrogen optimization by means of anaerobic digestion, and analyzing their results, recommendations for future experiments come to light. One of the most promising applications for the anaerobic digestion of waste substrates, or any organic substrate for that matter, is two-phase anaerobic digestion. In this scenario, first phase digesters produce hydrogen, while the second phase digesters produce methane. Produced methane gas is sparged through the first phase, increasing hydrogen yields, and producing a gas mixture of hydrogen and methane that, when combusted in an IC engine, reduces the amount of nitrogen oxides emitted (TerMaath, Skolnik, Schefer, & Keller, 2006).

In terms of digester operation, increased automation should be investigated. Gas sparging rate could potentially be automated to sustain consistent hydrogen

yields, an important factor for large scale hydrogen production. Despite some of the drawbacks to automation, continuous monitoring and control of bench scale anaerobic digestion systems could provide steady conditions resulting in higher confidence in the obtained. At a larger scale, one issue that could be detrimental to overall hydrogen production is the attached growth of methanogenic bacteria to the reactor walls. Long-term experiments should be run to investigate the health of the culture over time, determining the effect of possible methanogenic attached growths, and options for removing the growth if necessary. The costliest aspect of digester operation in this experiment was the phosphate buffer system. New sources of alkalinity should be studied to reduce overall operational costs to the digestion system while maintaining an adequate buffering capacity.

As waste substrates become more desirable and feasible for their use in fermentative hydrogen production, recalcitrant waste substrates treated with biogas enzymes should be studied. Biogas enzymes have the potential to break down more difficult substrates, decrease retention times while maintaining the same rate of fermentation, increase the quality of biogas, and even increase biogas production while using less feedstock (DuPont, 2016). Studies have shown that digesters that are fed biogas enzyme-treated substrate have reduced digester operational costs by 10% (Dupont, 2016). However, the cost and energy effectiveness of bio enzymes should be further studied.

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