EVALUATION OF ANAEROBIC BIODEGRADABILITY OF ZOOLOGICAL ORGANIC WASTE TO ENHANCE SUSTAINABLE WASTE MANAGEMENT AT ZOOS

By

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ABSTRACT

EVALUATION OF ANAEROBIC BIODEGRADABILITY OF ZOOLOGICAL ORGANIC WASTE TO ENHANCE SUSTAINABLE WASTE MANAGEMENT AT ZOOS

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Zoos across the country are pushing for sustainable solutions to mitigate their high-energy consumption and provide organic waste management. Anaerobic digestion provides an alternative to traditional waste management solutions such as landfilling, incineration, and composting. The Detroit Zoological Society (DZS) was selected as the zoo for this study. This study analyzed seven waste samples and mixtures from the DZS. A biochemical methane potential (BMP) test was performed on these wastes and their mixtures to determine the energy content. The results show that all samples are anaerobically biodegradable with samples yielding 501, 622, 269, 117, 232, 653, and 302 L biogas per kg initial VS for carnivore, primate, hoofstock mix, bird mix, zoo mix, food waste, and ZMF, respectively. The energy balance and carbon footprint analyses on BMP data further conclude that anaerobic digestion can efficiently handle zoo wastes, generate renewable energy to compensate 1.4% of the zoo's energy demand for their animal operations, as well as reduce carbon dioxide emission by 16%. In addition, the zoo mix and ZMF samples were tested in small-scale pilot digesters. Results show that the zoo mix sample yielded the highest cumulative gas production while the ZMF sample indicated inhibition due to low operating pH. The zoo mix and ZMF samples were fit to the Modified Gompertz Equation model to determine performance parameters for scale-up considerations.

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KEY TO SYMBOLS

AD	anaerobic digestion
ADREC	Anaerobic Digestion Research and Education Center
AZA	Association of Zoos and Aquariums
BMP	biochemical methane potential (L biogas per kg VS)
BMP_m	biochemical methane potential (L CH_4 per kg VS)
CH_4	methane
CN	carbon nitrogen
CO	carbon monoxide
CO_2	carbon dioxide
COD	chemical oxygen demand
CSTR	continuous stirred tank reactor
DZS	Detroit Zoological Society
GWP	global warming potential
H_2S	hydrogen sulfide
MSU	Michigan State University
MSUSCAD	Michigan State University South Campus Anaerobic Digester
N_2	nitrogen
NH_3	ammonia gas
NH_3-N	ammonia-nitrogen
OSHA	Occupational Health and Safety Administration
O_2	oxygen

RMSE	root mean square error
SSC	scaled sensitivity coefficient
TS	total solids
VS	volatile solids
ZMF	zoo mix & 10 % food waste

1. INTRODUCTION

1.1 Problem statement

Sustainability is becoming increasingly important for zoos and aquariums across the country. The Association of Zoos and Aquariums (AZA), with over 230 accredited zoos and aquariums in the United States, supports the conservation of animals, and providing resources for sustainable practices through green initiatives ("Association of Zoos and Aquariums," 2016). In 2008, the "Communicating Climate Change and the Oceans Summit" was held in Monterey, California (Kelsey, 2010). The results of the summit were a mobilization of representatives to make an effort to fight climate change through visitor education in aquariums (Kelsey, 2010). The same summit, held in Baltimore, Maryland in 2012, followed up on the 2008 conclusions and furthered the discussion on increasing climate change awareness through a variety of initiatives (*2012 Summit Aquariums Communicating Climate Change*, 2011). A public opinion survey conducted by The Ocean Project (2009) that found that zoos and aquariums are trusted public outlets for environmental action and campaigns for climate change, so that people expect information and leadership on climate change from these venues ("America, the Ocean, and Climate Change: New Research Insights for Conservation, Awareness, and Action," 2009; Kerr, 2010).

The Detroit Zoological Society (DZS) in Royal Oak, Michigan, began operations in 1928 with just 14 permanently housed animals, and is now home to over 2,400 animals ("Detroit Zoo," 2016). The AZA accredited zoo abides by the vision of AZA to promote green practices and holds paramount a commitment to environmental leadership and conservation ("Detroit Zoo," 2016; "Greenprint," 2016). As part of this commitment, the zoo developed an initiative, Greenprint, as a “green roadmap” to improve zoo policies and practices to decrease the zoo’s environmental footprint ("Greenprint," 2016). In addition to Greenprint, the zoo also has an initiative entitled “The Zoo that Could”, that promotes on-site energy production and the use of 100% renewable energy in the zoo ("The Zoo that Could,"). At the forefront of sustainability issues for the Detroit Zoo and other zoos across the world are organic waste management and high energy consumption (Klasson & Nghiem, 2003; Kusch, 2012).

The Detroit Zoological Society generates over 450 metric tons of animal manure each year. A majority of this waste is collected in a 20-yard garbage truck and hauled offsite to be composted (Handbury, 2016). Along with animal manure, each year the zoo has 45 metric tons of organic food waste generated from on-site cafeteria style food venues (Handbury, 2016).

In addition to the need for sustainable waste management, there is also a large energy consumption at zoos across the United States, with one study estimating 0.52-26.32 kWh per square meter per year (Kusch, 2012). Using this estimate, at nearly 506,000 square meters, the energy consumed by the Detroit Zoo is between 263,000 and 13,318,000 kWh per year. This wide range is attributed to the recreation of animal habitats with differences in energy requirements such as heating, cooling, and lighting (Kusch, 2012). Animal care management guidelines from AZA provide standards for animal habitats including temperature ranges, lighting, and water and air quality, thus setting baseline values for energy consumption ("Association of Zoos and Aquariums," 2016).

Anaerobic digestion (AD) is a technology that converts organic waste materials into value added products. AD provides appropriate waste treatment in an energy-efficient manner, while mitigating the adverse environmental impact of organic waste disposal. The outputs of anaerobic digestion are a nutrient rich digestate and biogas containing 55 to 75% methane (Tambone et al., 2010). Methane (CH_4), the same constituent in natural gas, can be converted to generate electricity or directly combusted for fuel. Digestate can be further treated with composting or other value added treatments, or land applied as an organic fertilizer.

There is limited information regarding characterization, biogas potential, and anaerobic digestion of zoo animal wastes (Kusch, 2012). Waste management practices at zoos across the United States include composting, landfilling, and incineration, all of which deny the potential to maximize value from the waste produced. In addition, landfilling and incineration of wastes can produce CH_4 and carbon dioxide (CO_2), noxious greenhouse gasses released directly into the environment contributing to global climate change. With an in depth understanding of organic waste produced at zoos, anaerobic digestion systems can be applied to alleviate adverse environmental impacts.

In addition, optimization, safety, education, and operations must also be considered to achieve maximum benefits of an anaerobic digestion system. Proper education on management is important in ensuring long term success of the system (Bracmort, Burns, Beddoes, & Lazarus, 2008).

1.2 Goal and objectives

In order to determine the treatment capacity of on-site organic waste including animal manure and food waste from the Detroit Zoo, this study performed a comprehensive analysis of zoo animal waste. The analysis was conducted to allow zoos to make educated decisions in design, implementation, and optimization of an anaerobic digester for on-site waste management. The specific objectives of this study were to:

(1) quantify and characterize organic waste substrates from the Detroit Zoo for application in anaerobic digestion,

(2) determine the potential biogas production from manure and food waste, generated from the zoo, and

(3) establish and improve the digester operating parameters such as feedstock for codigestion, retention time, and leachate recirculation schedule through small-scale pilot testing and model development.

2. LITERATURE REVIEW

2.1 Anaerobic digestion

Anaerobic digestion (AD) is a waste treatment technology that biologically converts organic waste into value added products that thrive in the absence of oxygen (Olsen & Caruana, 2011). There are four primary reactions in AD including (1) hydrolysis, (2) acidogenesis, (3) acetogenesis, and (4) methanogenesis (Crook & Gould, 2009; Khalid, Arshad, Anjum, Mahmood, & Dawson, 2011; Mani, Sundaram, & Das, 2016; Q. Zhang, Hu, & Lee, 2016). During the process complex organic compounds in the substrate are converted into methane-rich biogas (Brule, Oechsner, & Jungbluth, 2014; Crook & Gould, 2009). In addition to biogas, solid and liquid effluent, or digestate, are produced.

2.1.1 Factors influencing anaerobic digestion

Several important factors that are used to measure the quality of waste treatment and methane production in anaerobic digestion including pH, temperature, total solids (TS), volatile solids (VS), chemical oxygen demand (COD), carbon nitrogen (CN) ratio, and ammonia-nitrogen (NH_3-N).

VS and COD content are both parameters that can be used to analytically predict the biogas output of a substrate in anaerobic digestion, therefore determining the suitability of a substrate for digestion (Crook & Gould, 2009). During fermentation volatile solids are turned into acids and then used by methanogens to produce biogas (Brule et al., 2014; Crook & Gould, 2009). High volatile solids content is desirable for increasing gas production (Crook & Gould, 2009). In addition to substrates with high volatile solids content, wastewaters with COD greater than 250 mg/L are applicable for digestion given that there is a sufficient amount of organic material that is able to be used for digestion (Mes, Stams, Reith, & Zeeman, 2003; Olsen & Caruana, 2011). Under ideal temperature, microbial, and degradability conditions, each kg of COD converted can produce 331 L methane (Crook & Gould, 2009).

Temperature and pH are both parameters that need to be considered for the optimal methane production and stability of the microbial community in the digester. The pH of a digester is important to maintain viability of the anaerobic microbes in the material (Liu, Yuan, Zeng, Li, & Li, 2008). The pH effects the enzymatic activity in the digester given that certain enzymes are active in a narrow band of pH values (Lay, Li, & Noike, 1997). Low pH can prevent the production of methane and produce a buildup of hydrogen (H_2) which reduces pH further and inhibits production (Crook & Gould, 2009). One study developed a model for determining pH for optimum methane production at varying temperatures that can be useful in digester operation and optimization (Liu et al., 2008).

Carbon, a food for anaerobic microbes, is used up 25 to 30 times faster by microbes than nitrogen during anaerobic digestion the CN ratio should be in the 25:1 to 30:1 range for efficient digestion (Yadvika, Santosh, Sreekrishnan, Kohli, & Rana, 2004). In a study that observed differences in temperature ranges and CN ratio on anaerobic digestion of dairy (cow) manure, poultry manure, and rice straw, found a CN ratios of 25:1 and 30:1 at temperatures of 35°C and 55°C, respectively, yielded maximum methane production (Wang, Lu, Li, & Yang, 2014). The same study found that lower CN ratios of 15 and 20 at 35°C and 55°C reduced methane potential and increased ammonia inhibition (Wang et al., 2014). Ammonia-nitrogen can be used as a measure of the viability of the gas production in the digestion process. Ammonia toxicity can occur at ammonia concentrations above 3,000 mg/L and can cause digester failure (Crook & Gould, 2009).

Anaerobic digestion can occur in three different temperature ranges: psychrophilic (below 20°C), mesophilic (25 to 40°C), and thermophilic (52 to 58°C) (Crook & Gould, 2009; Donoso-Bravo, Bandara, Satoh, & Ruiz-Filippi, 2013). The mesophilic range, usually around 38°C, is considered to be the preferred temperature for stability of microorganisms (Crook & Gould, 2009). Donoso-Bravo, Bandara, Satoh, and Ruiz-Filippi (2013) compared two models on the effect of temperature on anaerobic digestion wherein the temperature was allowed to fluctuate with seasonal changes from 5-30°C (Donoso-Bravo et al., 2013). The study determined that a cardinal temperature model could accurately describe the trend in both CH_4 and CO_2 production, wherein temperature fluctuations directly impacted gas production (Donoso-Bravo et al., 2013).

2.1.2 Evaluation of substrates for anaerobic digestion

To determine the feasibility and economic viability of using a certain substrate for anaerobic digestion, there are several methods and method adaptations for estimating biogas potential of organic waste materials including: a Biochemical Methane Potential (BMP) test and an Automatic Methane Potential Test System (ASMPTS), among other methods (Badshah, Lam, Liu, & Mattiasson, 2012; El Achkar et al., 2016). BMP results are typically reported as L biogas per kg of volatile solid in the raw feed. Using the conversion method described by Maclellan, Chen, Kraemer, Zhong, and Liao (2013), the biogas potential value can be converted to estimated electrical output (Maclellan et al., 2013). For the purpose of evaluating zoo organic waste materials, this study will employ the BMP test method described by Faivor and Kirk (2011), and derived from Chynoweth and others (1993) (Chynoweth, Turick, Owens, Jerger, & Peck, 1993; Faivor & Kirk, 2011). This BMP method uses a 2:1 sample VS to filtrate VS ratio for test set up. The test is run under temperature controlled mesophilic conditions. The results of the BMP test are the biogas potential (BMP), or methane potential (BMP_m).

There is a variety of organic feedstocks applicable to AD including manure and food waste, among other organic materials. Currently, there is significant research surrounding anaerobic digestion of ruminant hoofstock animal manure such as cattle, sheep, and goat. In addition, there is also prevalent research on other livestock animal wastes like horse, swine, and poultry litter. In a BMP study performed on five different livestock animal manures including: dairy (cow), swine, goat, and horse gas production was 295, 495, 242 and 222 L biogas per kg VS, respectively (Kafle & Chen, 2016). The Association for Technology and Structures reported BMP values for cattle, swine, poultry, as 420, 817, and 584 L biogas per kg VS, respectively ("Cost-Effectiveness Biogas Calculator," 2016). Another study reported BMP_m for cattle, swine, and poultry manure as 323, 558, and 290 L CH_4 per kg VS respectively (Hidalgo & Martín-Marroquín, 2015). It is expected that the BMP and characterization of animal waste at the Detroit Zoo, which contains 95% hoofstock animals, would be in the range of other hoofstock animals waste.

Codigestion is when two or more substrates are blended together to improve biogas production, digestion of the material, and economic feasibility. Prior to excretion, animal manure goes through the digestive tract of the animal, therefore utilizing much of the energy potential, whereas food waste has not previously undergone digestion and therefore has higher energy content (López, Passeggi, & Borzacconi, 2015). Food waste could be codigested with the zoo waste to improve gas production. However, food waste as a mono-substrate is not favorable for anaerobic digestion due to its rapid biodegradability, production of long chain fatty acids, and drop in pH throughout the course of the test (Ebner, Labatut, Lodge, Williamson, & Trabold, 2016; C. Zhang, Xiao, Peng, Su, & Tan, 2013).

At the DZS, food waste can be codigested along with the herbivore animal waste to capitalize on synergistic properties of microbial communities in different waste streams. In a study performed by Zhang, Xiao, Peng, Su, and Tan (2013) the addition of food waste to cattle manure in comparison to manure digestion, in both batch and continuous tests, increased methane production (C. Zhang et al., 2013). Ebner, Labatut, Lodge, Williamson, and Trabold (2016) reported BMP_m values for manure and food waste ranging from 165 L CH_4 per kg VS for a blend of preparation waste (kitchen waste with low biodegradability) to 496 L CH_4 per kg VS for a food service blend (food preparation post-consumer waste, and uneaten food) (Ebner et al., 2016). The same study reported an increase in biogas potential in blends of raw manure and food waste, validating the benefits of codigestion with food waste (Ebner et al., 2016). Another report estimated BMP for food waste to be 879 L biogas per kg volatile solids ("Cost-Effectiveness Biogas Calculator," 2016).

2.1.3 Anaerobic digestion outputs

The products of anaerobic digestion are biogas and solid and liquid effluent (Wedwitschka, Jenson, & Liebetrau, 2016). Biogas containing CH_4, CO_2 and trace amounts of other gasses (ammonia (NH_3), hydrogen sulfide (H_2S), oxygen (O_2), nitrogen (N_2), and carbon monoxide (CO)) is produced as a result of anaerobic digestion (Khalid et al., 2011; Sun et al., 2015; Q. Zhang et al., 2016). Biogas with a composition of 55 to 75% methane has an energy content of 6.0 to 6.5 kWh per m^3 of biogas and can be combusted (Deublein & Steinhauser, 2008)

Biomethane is another term that is used to describe the methane portion of the biogas. In large scale anaerobic digestion systems biogas can be collected and upgraded to run a generator, but also can be sent to the natural gas grid or used in boilers and stoves (Sun et al., 2015).

Depending on the end use of the biomethane, it is usually necessary to improve the quality of the biogas to remove moisture, H_2S, and CO_2 (Sun et al., 2015). There are a variety of methods for upgrading biogas including water scrubbing, physical and chemical absorption, and membrane technology, among others (Sun et al., 2015).

As suggested by Appels and others (2011), the digestate, or slurry produced from anaerobic digestion can separated and land applied as a fertilizer or turned into other value added products such as biochar or bioalcohol (Appels et al., 2011; Kondaveeti & Min, 2015; Tambone et al., 2010). Digestate from anaerobic digestion is high in mineralized nutrients, or nutrients that are readily used by crops, therefore posing a positive economic reuse of waste material (Crook & Gould, 2009; "Local energy production from biowaste," 2014). In batch anaerobic digestion, liquid digestate can be recycled to inoculate the new material (Wedwitschka et al., 2016).

2.1.4 Environmental impact of anaerobic digestion

If released into the environment directly, as in a landfill, methane gas can have adverse environmental impacts and increase the global warming potential (GWP). GWP refers to how much energy one ton of gas will absorb compared to one ton of CO_2 over time ("Understanding Global Warming Potentials," 2016). As reported by the Environmental Protection Agency (EPA), CH_4, the primary component of biogas, has a GWP of 28-36 tons of CO_2 over a 100-year span ("Understanding Global Warming Potentials," 2016). CH_4, when combusted in a generator, produces CO_2 that is carbon-neutral, with less net impact on greenhouse emissions than direct release of biomethane (Khalid et al., 2011; Ward, Hobbs, Holliman, & Jones, 2008).

While digestate from AD can function as a well-mineralized fertilizer for crop application, it can also adversely impact the environment if it is not utilized properly. Digestate can be applied in excess of what crops can uptake and can leach into local waterways causing eutrophication (Yilmazel & Demirer, 2013). One study suggested that removal of the nitrogen and phosphorus content in the digestate is necessary to avoid eutrophication (Yilmazel & Demirer, 2013). The appropriate level of nitrogen and phosphorus needs to be reduced during digestion or post-digestion to avoid adverse environmental impact from over applying the material. With a nutrient loading calculation of the targeted land, digestate can be appropriately land applied as an organic fertilizer to reduce the use of chemical based fertilizers.

2.1.5 Locations and types of anaerobic digesters

Anaerobic digesters have applications in many different parts of society and the world. In small scale application, digesters can function to improve energy reliability and deal with waste treatment roadblocks, such as those in rural India (Appels et al., 2011). Agriculture and industry are also important applications for anaerobic digestion as many digesters in the United States are located on dairy farms, wastewater treatment plants, and food processing facilities (EPA, 2016; Water, 2016).

There are several different designs of digesters that consider varying design constraints such as manure handling on-site, TS content of material, material consistency, and local climate (Roos, Martin Jr., & Moser, 2004). Covered lagoon and fixed film reactors require a solids content of less than 3% and are suitable in warm and temperate climates and is generally applied in dairy and swine applications (Crook & Gould, 2009; Roos et al., 2004). A continuous Stirred Tank Reactor (CSTR), also known as a complete mix digester, is common in dairy and swine operations with a total solids concentration of manure less than 11% (*Conservation Practice Standard Anaerobic Digester Code 366*, 2009; Crook & Gould, 2009; Roos et al., 2004).

Organic waste produced from the zoo is high in solids content due to the manure being mixed with bedding, leaves, animal feed, and other organic materials. Studies report high solids digesters are applicable when feedstocks have a total solids content greater than 25-30% (w/w) (Mes et al., 2003; Wedwitschka et al., 2016) . Given the high solids content of zoo organic waste, high solids batch anaerobic digestion is the best suited technology (Kusch, 2012; Wedwitschka et al., 2016). In a high-solids batch reactor the substrate is placed into a chamber where it remains, typically without mixing, for 20 to 30, or more days (Wedwitschka et al., 2016). Batch digestion relies on recirculation of liquid digestate, or leachate, from the previously digested material to provide a microbial inoculum to each new batch (Wedwitschka et al., 2016). The leachate percolates through the material, is collected in a holding tank, and pumped back into the chamber. Solid digestate is removed after the required retention time and can be further composted or land applied.

High-solids digestion generally does not need water added to the process, and is simple to construct and relatively inexpensive to operate (Wedwitschka et al., 2016). High-solids digesters are favorable for integration with a composting process, as the effluent does not have to pass through a solid liquid separator. Given that the digestate does not have to be separated, and that the material is not mixed regularly as in a CSTR, there is less energy consumption from these types of digesters. Despite less energy consumption, there is typically less energy produced, as the system is generally not as efficient as CSTR systems likely due to less uniformity in the process (Wedwitschka et al., 2016). As high solids-digesters require recycle of solid digestate to ramp up production and stabilize the new system, often the capacity must be large to accommodate the material needed, which can increase construction costs.

High solids batch digestion systems are dependent on operational parameters being optimal for digestion efficiency. Retention time, leachate recirculation, and feedstock codigestion are all parameters that can be adjusted to improve digester performance and biogas production. Small-scale pilot systems can be employed to study the digestion performance and develop models for methane production to predict scale-up performance parameters.

2.1.6 Models for anaerobic digestion

Modeling allows for an improved understanding of anaerobic digestion, increased knowledge of design constraints, and a method for prediction of waste treatment capacity and methane production (Garcia-Ochoa, Santos, Naval, Guardiola, & Lopez, 1999) . There are several models for batch anaerobic digestion of livestock manures and other organic materials. One study was able to successfully model the production of acetogenic and methanogenic bacterial biomass (Garcia-Ochoa et al., 1999). By simplifying the steps of anaerobic digestion into acid formation (hydrolysis and acidogensis), and methane formation (acetogensis and methanogensis), Brule and others (2014) developed a model for the prediction of methane production from a BMP test (Brule et al., 2014). Another study was able to optimize the design time of leachate recirculation by determining a relationship between the sprinkling rate of leachate and the solids retention time to maximize methane production (Thamsiriroj, Nizami, & Murphy, 2012). One study comparatively analyzed the following models: the Logistic function, Modified Gompertz equation (Equation 20), and reaction curve-type model by fitting data from anaerobic digestion of sewage sludge (Donoso-Bravo, Pérez-Elvira, & Fdz-Polanco, 2010). The study found that each of the models were able to estimate the performance parameters. Another study used the same model to assess the performance conditions under influences of pH and moisture content in high solids sludge digestion (Lay et al., 1997). Appels et. al. (2011) concludes that more development of models is necessary to improve the understanding of the system for optimization (Appels et al., 2011).

2.2 Anaerobic digestion for zoos

As part of the Greenprint initiative, the DZS committed to “lessening their environmental impact” by developing a plan to “refine and improve daily practices, develop new policies and programs, and improve green literacy” ("Greenprint," 2016). As part of this initiative, the zoo looked to anaerobic digestion to provide a value added waste management solution to their on-site organic waste production.

2.2.1 Organic waste management at zoos

There is a need for sustainable waste management of organic material produced on-site at zoos. The Detroit Zoo produces over 450 metric tons of animal manure each year that is hauled offsite each week with a standard garbage truck to be composted. In addition, the zoo landfills 45 metric tons of organic food waste yearly. Anaerobic digestion is a viable solution to address organic waste management, while providing the economic incentive of waste reutilization. In order to determine the feasibility of an anaerobic digestion system at the Detroit Zoo, a comprehensive understanding of the organic waste is necessary.

Currently, composting is an option for management of the bird and hoofstock animal waste on site. Other animal manures in the primate, carnivore, and omnivore categories are landfilled due to concern of the end compost retaining human transmittable pathogens. According to Martins and others (2013), composting zoo animal waste is a good method to break down the waste (Martins et al., 2013). Although composting is an appropriate method for treatment, it does not allow biogas to be collected, which can improve the economic impact of organic waste management.

Anaerobic digestion is used for waste management for few zoos in the world, including the Hellabrun Zoo in Munich, Germany, and since 2017, the DZS. At the Munich Zoo, a batch anaerobic digester with 3 digestion chambers was constructed and operates on herbivore manure, bedding, and green wastes (Kusch, 2012). Each chamber has a loading capacity of 100 m^3 and average 410 m^3 per day of biogas under mesophilic conditions (Kusch, 2012). Biogas from the digester is ran through a combined heat and power generator to provide electricity and heat on site (Kusch, 2012). Although the Hellabrun Zoo has different animals than the Detroit Zoo, it validates that anaerobic digestion is feasible at zoos across the world.

2.2.2 Zoo animal waste characterization

There is minimal information available to describe the waste characteristics of zoo animal manure (Kusch, 2012). In a study performed by Oak Ridge National Laboratory, elephant and rhinoceros manure were determined to have a biogas potential of 26 L biogas per kg waste and 33 L biogas per kg waste, respectively. The study determined that an anaerobic digester that treats 20 metric tons of material per week would produce enough energy to run two standard garden grills. Due to limited characterization data, additional characterization of zoo animal wastes was necessary to determine the biogas potential and feasibility of a batch digester at the DZS.

2.2.3 Zoo energy consumption

In a study performed on German, Swiss, and Austrian zoos, and reported by Kusch, zoos consume 0.52 to 26 kWh per m^2 per year (also estimated to be 26 to 1,978 kWh per animal per year) (Kusch, 2012; Simon). There is a large variation in habitat energy consumption due to the different conditions that zoos must replicate for the animals to thrive in and the zoos use of interactive and visual displays (Kusch, 2012). The Detroit Zoo specifically, has 506,000 m^2 of ‘naturalistic habitats’, which converts to 263,000 to 13,300,000 kWh per m^2 land surface area per year ("Detroit Zoo," 2016; Kusch, 2012).

2.3 Anaerobic digester safety

To manage an anaerobic digester at a zoo, it is important to understand the safety aspects. The Occupational Safety and Health Administration (OSHA) regulations applicable to anaerobic digesters need to be implemented to maintain the safety of persons in contact with the digester.

Both influent and effluent from anaerobic digestion should be handled properly to avoid adverse impacts. Feedstock and digestate should be handled carefully and extra attention should be given when loading and unloading digester cells (Agstar, 2011). Material should be contained to the loading area and spills outside the area should be contained. Additionally, leachate is produced in the batch anaerobic digestion process and generally stored in a holding tank. Confined space training is required by OSHA for small spaces where workers must enter to perform tasks, such as fixing sump levels or clogged pipes that can occur in batch digestion (Agstar, 2011). Persons entering a confined space must test the atmosphere prior to entry using a handheld device, and O_2 should be above 19.5 percent volume by air, CH_4 below 5 percent volume by air, and H_2S level below 20 ppm (Agstar, 2011).

Given that anaerobic digestion produces a flammable gas, there are safety concerns that need to be addressed in operation. One primary concern is the anaerobic conditions within the digester. If air is mixed in large quantities, it may produce an explosive mixture of gas (Crook & Gould, 2009). The gas produced also has potential to leak to the environment causing a fire hazard, explosion potential, and an increase in GHG emissions (Crook & Gould, 2009). In a dry digestion system, there is risk when opening the chamber to refill it and being exposed to CH_4, CO_2, and H_2S. These gases produced with storage of organic material and during anaerobic digestion are asphyxiants and should be monitored closely (Agstar, 2011). Given this, it is important that operators always have a wearable safety monitor on-person and follow appropriate loading and unloading procedures.

The generator used for anaerobic digestion is a source of noise in anaerobic digestion. Proper noise canceling equipment should be employed to protect individuals from excessive noise. OSHA requires that the managing facility provide hearing protection to maintain a safe maximum allowable decibel level(Agstar, 2011). There are hazards associated with production of electrical generation. Licensed electricians should provide maintenance and repairs (Agstar, 2011). Electrical equipment should be regularly inspected and problems should be noted and fixed by licensed personnel (Agstar, 2011). Signage should be posted for electrical generation hazards present (Agstar, 2011).

An emergency action plan should be implemented in the AD facility that should include the events needed in case of an emergency at the facility (Agstar, 2011). Contact personnel, state and local health requirements, and equipment manuals should also be included. Emergency and safety equipment should be readily available on-site for operators to employ.

3. MATERIALS AND METHODS

3.1 Organic waste collection and handling

Organic waste samples were collected and analyzed by the MSU Anaerobic Digestion Research and Education Center (ADREC).

3.1.1 Zoo organic waste

Zoo manures were picked up triweekly from the DZS and subsamples were taken between April 2016 and September 2017. In addition to raw animal manure, the samples contained bedding, hay, leaves, twigs, and animal feed, among other organic materials. Samples were transported to MSU in coolers with ice. All zoo wastes were refrigerated at 4°C prior to analysis.

3.1.2 Food waste

Pre-consumer food waste used was collected from Brody Cafeteria at MSU and was assumed similar in nature to the cafeteria-style pre-consumer food waste generated at DZS. The waste was collected from a pulper containing all food prep wastes from the cafeteria. Due to the rapid degradability of food waste, samples were stored in a freezer at -18°C prior to analysis.

3.1.3 Filtrate

Filtrate was collected from the MSU South Campus Anaerobic Digester (MSUSCAD). The MSUSCAD utilized a mix of approximately 50% dairy manure and 50% food waste and food processing residuals as feedstock. The filtrate is the liquid portion of the effluent after the material passed through a screw press solid-liquid separator with a 500-micron main screen and 750-micron press screen. New filtrate was collected prior to each round of BMP samples (n=3) and weekly for pilot testing. Filtrate for characterization and BMP analyses was stored in a refrigerator at 4°C. Filtrate used in pilot testing was stored at room temperature, 20-22°C, opposed the refrigerator in order to mimic zoo conditions.

3.2 Waste quantification

The waste was quantified based on estimates provided from the DZS landscape and sustainability managers. Total waste production was calculated based on an estimated amount of cans picked up per animal habitat per pickup and the assumed density of the wastes. Given the large quantity of bedding used and the nature of the samples, the density of the hoofstock mix was assumed to be similar to sawdust, 272 kg/m^3, and bird mix was assumed to be the same as loose straw, 40 kg/m^3 (Glover, 1995; Lorimor, Powers, & Sutton, 2004). Waste production and percentages from the respective animal habitats can be found in Table 1. In addition to animal wastes, the DZS estimates 10 % of their total annual waste is food waste from their cafeterias and animal feed prep.

Table 1: Type and quantity of animal wastes at the Detroit Zoo

Animal category	**Animal type**	**Waste production (kg per year)**	**Weight percentage of the total waste (%)**
Hoofstock	Aardvark	13,752	2.57
	Barnyard [a]	85,950	16.06
	Bison	34,380	6.42
	Camel, Deer	85,950	16.06
	Eland	27,504	5.14
	Giraffe	41,256	7.71
	Guanaco, Rhea, Deer	27,504	5.14
	Kangaroo	20,628	3.85
	Rhino	68,760	12.85
	Veldt [b]	103,140	19.27
Bird	Bird Mix 1 [c]	5,056	0.94
	Bird Mix 2 [d]	5,056	0.94
	West Pampas [e]	1,011	0.19
Carnivore	Lion	9,412	1.76
Primate	Great Ape	5,772	1.08
Total		**535,131**	**100**

a. Barnyard includes cow, horse, swine, and goat.
b. Veldt includes warthog and zebra.
c. Bird mix 1 includes flamingo, vulture, golden crown, spoonbill, and stork.
d. Bird mix 2 includes flamingo, goose, and vulture.
e. West pampas includes emu and flightless birds.

3.3 Preparation of sample mixtures

Animal wastes were grouped into four categories including: carnivore, primate, hoofstock mix, and bird mix. The carnivore and primate samples contained the lion and great ape habitats, respectively. Hoofstock and bird mixtures were prepared according to the estimated waste production from the respective category (Figures 1a and 1b). A zoo mix was prepared by mixing the carnivore, primate, hoofstock mix, and bird mix based on the estimated proportion of waste produced each year (Figure 1c). Additionally, Based on food waste estimates from the DZS, the zoo mix was combined with food waste in a 90:10 ratio of zoo mix to food waste (ZMF) (Figure 1d). For pilot testing and NREL characterization, carnivore and primate wastes were excluded due to the likelihood of them not being included in the commercial-scale system at the Detroit Zoo. All other testing included carnivore and primate wastes.

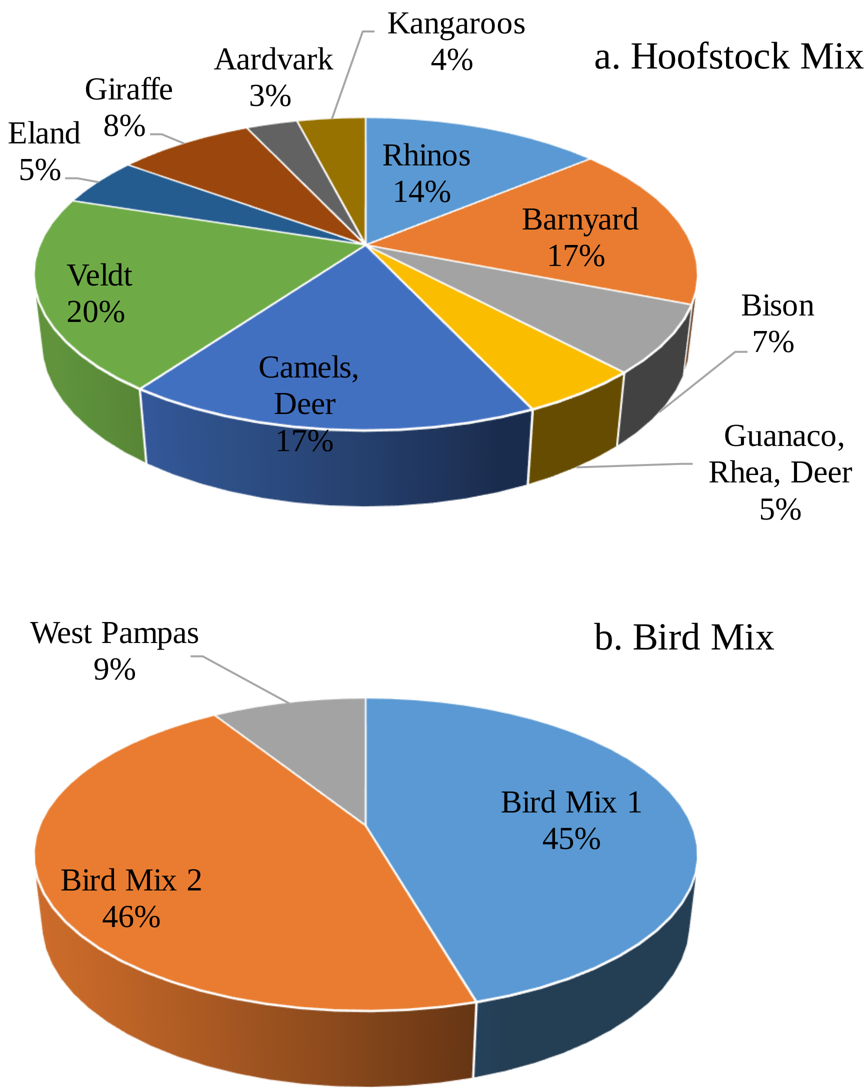
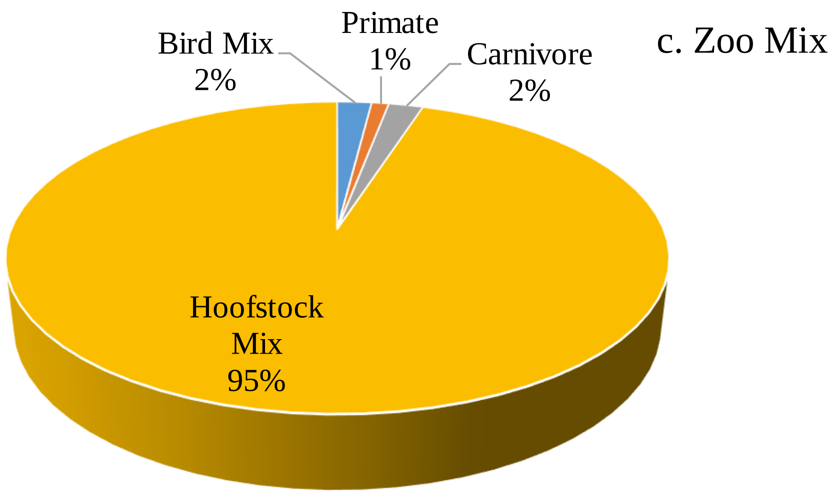
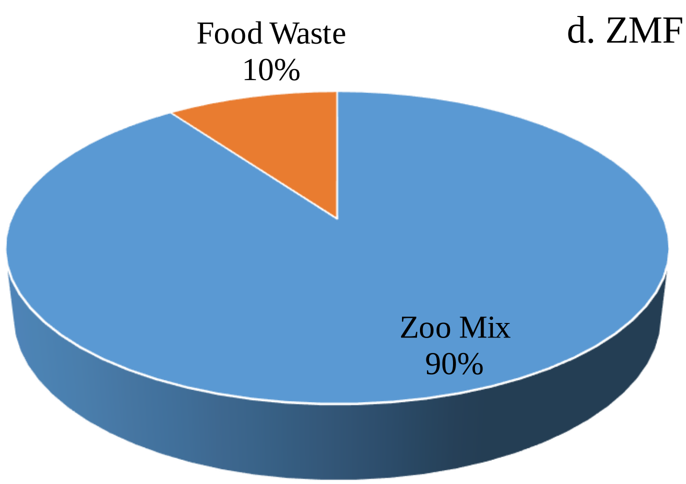


Figure 1: Weight distribution of different waste blends

Figure 1 (cont'd)





3.4 Waste characterization

The raw samples were characterized for TS and VS using EPA accepted Hach methods 8271 and 8276, respectively. For TS, the oven holding time was increased from six to 24 hours to ensure complete drying. The VS holding time was increased from one to 6 hours to guarantee complete sample combustion.

Pre and post BMP digestion analyses performed were TS, VS, NH_3-N, CN ratio, COD, and pH. NH_3-N was performed using accepted Environmental Protection Agency (EPA) Hach standard 10205. COD was performed using EPA accepted Hach method 8000. The BMP pH and EC was tested using an Accumet Excel XL60 meter by Fisher Scientific.

Pre and post pilot digestion testing included TS, VS, CN ratio, and structural carbohydrates and lignin. CN ratio was performed by two outside laboratories including Michigan State University (MSU) Plant and Soil Science Lab and A&L Great Lakes Laboratories using elemental analyses. Samples sent to an outside lab for CN ratio analysis were stored in containers with ice during transport and were shipped overnight. The pilot leachate pH testing was done using a Thermo Scientific Orion Star A215 Benchtop meter.

3.5 Biochemical methane potential Test (BMP)

Three rounds of BMP testing were performed. Each round, new zoo, food waste, and filtrate samples were collected. Samples were collected in June, July, and October of 2016. The samples tested were carnivore, primate, hoofstock mix, bird mix, zoo mix, food waste, and ZMF.

3.5.1 Set-up

The BMP samples were set up using the method described by Faivor and Kirk (2011), and derived from Chynoweth and others (Chynoweth et al., 1993; Faivor & Kirk, 2011). After the initial raw sample characterization was completed, sample blends were calculated for the BMP analysis based on a 2:1 filtrate to sample ratio, with the volume of the filtrate not to exceed 20% of the bottle volume. Blends were set up to contain 60 mL of filtrate, a calculated amount of sample to achieve a 2:1 filtrate to sample VS ratio, and the remaining amount up to 300 mL of deionized water. Due to the heterogeneity of the DZS samples, they were macerated using a Nutri-Ninja Professional BL450 900 Watt blender without the addition of water to increase uniformity (Hansen et al., 2004). BMPs for each sample were set up in triplicate to account for varying quality of filtrate and heterogeneity of the material (Hansen et al., 2004). Triplicate controls were also set up containing 60 mL of filtrate and 240 mL of deionized water. The blends were mixed on a stir plate for 10 minutes and 150 mL of the blend was placed into a 200 mL Kimble Chase serum bottle. The remaining 150 mL of the blends were retained for pre-digestion analyses. The bottles were sealed with a butyl rubber septa from Geo-Microbial Technologies, INC. and a crimped aluminum cap. The bottles were flushed with nitrogen at a flowrate of 750 mL per minute for 10 minutes and placed into a mesophilic temperature room at 35°C on a Thermolyne Bigger Bill Oscillator laboratory mixer. After two hours, gas was released from the bottles and the time was recorded as the starting time. The BMP testing continued for 30 days.

3.5.2 Operation and Monitoring

Gas production was measured daily using a 10, 30, 50 or 100 mL wetted glass syringe. Syringe volume selection was based on the prior day's gas production. Gas composition including CH_4, CO_2, and H_2S was measured weekly using a HayeSep D column in a SRI 8610 Gas Chromatograph with a flame ionization detector (FID) and thermal conductivity detector (TCD). The sample was taken from the bottle headspace after gas production was measured.

The bottles were taken apart after 30 days and post-digestion analyses were performed on the effluent.

3.5.3 BMP Calculations

Raw gas is measured in a lab maintained at 22°C and is assumed saturated. Gas is normalized for standard temperature (0°C) and pressure (1 atm) (STP) using the Equation 1.

$$G_{STP} = G_R \times 0.897 \tag{1}$$

G_{STP}	gas normalized for standard temperature and pressure, mL
G_R	raw gas production, mL
0.897	STP conversion factor for conditions in East Lansing, MI

Each bottle's biogas production is normalized to the control bottles that contain only filtrate and DI water using Equation 2.

$$G_N = G_{STP} - \frac{Control_1 + Control_2 + Control_3}{3} \tag{2}$$

G_N	normalized gas production, mL
$Control_1$	biogas production from control 1, mL
$Control_2$	biogas production from control 2, mL
$Control_3$	biogas production from control 3, mL

The VS content is calculated for the bottles based on the VS of the raw sample using Equation 3.

$$VS_N = VS_R \times S \times \frac{1}{1000} \tag{3}$$

VS_N	volatile solids content in the bottle, mg
VS_R	volatile solids content of the raw sample, mg/kg
S	mass of sample in the bottle, g
1/1000	conversion factor, kg/g

The biogas content of the respective bottles (BMP_i) was found by using Equation 4.

$$BMP_i = \frac{G_N}{VS_N} \tag{4}$$

i	bottle number

The triplicate bottles are then averaged using Equation 5.

$$BMP = \frac{BMP_1 + BMP_2 + BMP_3}{3} \times \frac{1}{1000} \times 10^6 \tag{5}$$

BMP	biochemical methane potential, L biogas/kg initial VS
1/1000	conversion factor, L/mL
10^6	conversion factor, mg/kg

3.6 Mass and Energy Balance

Mass and energy balance analysis were carried out based on the experimental data and local environmental conditions in Detroit, MI. The analysis was conducted based on 1 kg dry raw feed. The BMP test data were used to carry out the analysis. The CH_4 production was calculated using Equation 6.

$$M = \frac{BMP_m \times G \times 16}{0.082 \times T} \tag{6}$$

M	CH_4 production, g methane/kg dry feed
BMP_m	biochemical methane potential, L methane/kg initial VS
G	ratio of initial VS to TS in the raw feed
T	biogas temperature, K
16	molecular weight of methane, g/mol
0.082	gas constant, L atm/K/mol

The energy balance was analyzed based on high heat value (HHV) of methane, local temperature, and thermal efficiencies of combined heat and power (CHP) unit. Energy inputs and outputs were assigned as negative and positive, respectively. The biogas was assumed to be used by a combined heat and power (CHP) unit to generate heat and electricity. The energy outputs as heat (E_{heat}) and electricity ($E_{electricity}$) were calculated using Equations 7 and 8, respectively.

$$E_{heat} = M \times 55 \times 0.6 \times 0.0002778 \tag{7}$$

$$E_{electricity} = M \times 55 \times 0.3 \times \frac{1}{3600} \tag{8}$$

E_{heat}	energy output as heat, kWh-e/kg dry raw feed
$E_{electricity}$	energy output as electricity, kWh-e/kg dry raw feed
55	HHV of methane, kJ/g
0.6	thermal efficiency of a typical CHP (Kurchania, Panwar, & D. Pagar, 2011)
0.3	electrical efficiency of a gas engine
1/3600	conversion factor, kWh/kJ

The energy inputs for the digestion operation include heat to maintain the digestion temperature as well as electricity to power pumps, mixers, and other accessary equipment. The heat and electricity energy inputs were calculated using Equations 9 and 10, respectively.

$$W_{heat} = \frac{1}{TS_{Feed}} \times C_p \times (308.16 - 283.16) \times (1 + 10\%) \times 0.0002778 \tag{9}$$

$$W_{electricity} = E_{electricity} \times 9\% \tag{10}$$

W_{heat}	heat input, kWh-e/kg dry raw feed
$W_{electricity}$	electricity input, kWh-e/kg dry raw feed
TS_{Feed}	TS content of the feed, %
C_p	specific heat capacity of the wet feed, 3.95 kJ/kg/K (Zhong et al., 2015)
308.16	digestion temperature, K

283.16	average atmosphere (feed) temperature in Detroit, MI, K
10%	percentage of the parasitic heat to maintain digester temperature aside from heat required by the feed
9%	percentage of the electricity required to power digester related equipment (Sliz-Szkliniarz & Vogt, 2012)

3.7 Carbon Footprint

The carbon footprint analysis focused on three main sources of carbon dioxide emission: electricity usage, natural gas usage, and waste handling. Carbon dioxide equivalent emissions from electricity and natural gas usages (EPA, 2017) were calculated using the EPA methods in Equations 11 and 12, respectively.

$$CO_{2e-electricity} = Electricity_{annual} \times 0.6997 \tag{11}$$

$$CO_{2e-gas} = Heat_{annual} \times 3600 \times 50.25 \times 1/10^6 \tag{12}$$

$CO_{2e\text{-}electricity}$	CO_2 equivalent emissions from electricity (kg/year)
$CO_{2e\text{-}gas}$	CO_2 equivalent emissions from natural gas (kg/year)
$Electricity_{annual}$	annual electricity consumption at the zoo (kWh-e/year)
0.6997	CO_2 equivalent emission per kWh electricity usage (kg/kWh) (EPA, 2014)
$Heat_{annual}$	thermal energy from natural gas consumption at the zoo (kWh-e/year)
3,600	conversion factor, kJ/kWh
50.25	CO_2 equivalent emission per kJ thermal energy use, mg/kJ (EPA, 2017)

$1/10^6$	conversion factor, kg/mg

Two waste handling processes of composting and landfill are applied in these analyses to study the impact of them on carbon footprint of the zoo. The EPA and IPCC methods (EPA, 2010b) were modified and applied to estimate CO_2 equivalent emissions. The CO_2 equivalent emission of the landfill was determined using Equation 13.

$$CO_{2e-landfill} = 21 \times F_{wastes} \times VS_{wastes} \times BMP_m \times 0.60 \times \frac{1}{1000} \times 0.67 + 1 \times F_{wastes} \times VS_{wastes} \times (BMP - BMP_m \times 0.60) \times 1/1000 \times 1.77 \quad (13)$$

$CO_{2e\text{-landfill}}$	CO_2 equivalent landfill admission, kg/year
21	CH_4 GWP
1	CO_2 GWP
F_{wastes}	annual zoo waste generation (zoo mix and food waste), kg wet wastes/year
VS_{wastes}	VS of wastes, kg VS/kg wet wastes
0.60	landfill gas collection efficiency (EPA, 2010a)
1/1000	conversion factor, kg/g
1.77	conversion factor of m^3 to kg for CO_2
0.67	conversion factor of m^3 to kg CH_4

The CO_2 equivalent emission of the in-vessel composting was calculated using Equation 14.

$$CO_{2e-composting} = 21 \times F_{wastes} \times VS_{wastes} \times BMP_m \times 0.005 \times \frac{1}{1000} \times 0.67 + F_{wastes} \times VS_{wastes} \times (BMP - BMP_m \times 0.005) \times \frac{1}{1000} \times 1.77 + F_{wastes} \times 0.1 \times 0.6997 \tag{14}$$

0.005	CH_4 conversion factor for in-vessel composting (EPA, 2010b)
0.1	electricity consumption rate of composting operation (kWh/kg wet wastes) (H. Zhang & Matsuto, 2011)

The implementation of anaerobic digestion with biogas power generation converts the CH_4 in the biogas into CO_2. The CO_2 emissions from anaerobic digestion were calculated using Equation 15.

$$CO_{2e-ad} = F_{wastes} \times VS_{wastes} \times BMP \times 1/1000 \times 1.77 \tag{15}$$

CO_{2e-ad}	CO_2 emissions from anaerobic digestion, kg/year

The total CO_2 equivalent emission of the zoo with landfill treatment of zoo wastes was calculated using Equation 16.

$$CO_{2e-zoo,landfill} = CO_{2e-electricity} + CO_{2e-gas} + CO_{2e-landfill} \tag{16}$$

$CO_{2e-zoo,landfill}$	CO_2 equivalent zoo emissions with landfill treatment, kg/year

The total CO_2 equivalent emission of the zoo with composting treatment of zoo wastes was calculated using Equation 17.

$$CO_{2e-zoo,composting} = CO_{2e-electricity} + CO_{2e-gas} + CO_{2e-composting} \quad (17)$$

$CO_{2e\text{-zoo, composting}}$ CO_2 equivalent zoo emissions with composting treatment, kg/year

The total CO_2 equivalent emission of the zoo with anaerobic digestion of zoo wastes was calculated using Equation 18.

$$CO_{2e-zoo,ad} = CO_{2e-electricity} + CO_{2e-gas} + CO_{2e-ad} \quad (18)$$

$CO_{2e\text{-zoo,ad}}$ CO_2 equivalent zoo emissions with AD treatment, kg/year

3.8 Pilot systems design and operation and analysis

Pilot systems were designed to test operational parameters of batch AD for organic waste from the DZS. High solids batch AD pilots were built based on the design parameters of the dry anaerobic digester constructed at the Detroit Zoo. The design conditions for both the DZS digester and the pilot scale digester are found in Table 2. A diagram of the pilot digester design in shown in Figure 2. A list of materials used for the construction of the small-scale pilot digesters is included in Table 3.

Table 2: Comparison of design conditions for Detroit Zoo digester and pilot digesters

Detroit Zoo Digester		**Small-scale Pilot Digester**	
Construction Material	Concrete	Construction Material	PVC
Height (m)	3.0	Height (m)	0.3
Length (m)	4.3	Radius (m)	0.08
Width (m)	3.0		
Batch (kg)	9,100	Batch (kg)	1.27

Table 3: List of materials used for construction of small-scale pilot digesters

Component	Quantity	Material	Part Number
Flange socket end[a]	1	PVC	4881K221
Flange cap[a]	1	PVC	4881K972
End cap[a]	1	PVC	4880K141
Oil-resistant Buna-N Gasket[a]	1	Buna-N rubber	8516T243
Schedule 40 PVC pipe[a]	1	PVC	48925K25
Barbed hose fitting[a]	4	brass	5346K19
Ball valve[a]	3	PVC	45975K28
Thick wall 0.95 cm tee fitting[a]	1	PVC	4596K322
Pressure gauge[a]	1	Brass	4026K17
Hex nipple[a]	3	Brass	5485K23
Tubing[a]	2 m	vinyl	5233K63
Wet tip gas meter[b]	1	-	-

a. ("McMaster-Carr," 2018)

b. (wettipgasmeter.com, 2018)

Unlike the DZS digester, the pilot systems were cylindrical in shape due to the ease and cost-effectiveness of sourcing polyvinyl chloride (PVC) piping. The body of the digester was a 0.30 m long schedule 40 PVC pipe. A PVC flange socket end was affixed on top of the digester body. An oil-resistant compressible Buna-N gasket was placed on the flange socket and a flange cap was bolted down using eight 19.05 mm bolts. A standard-wall PVC pipe fitting was affixed to the bottom of the digester.

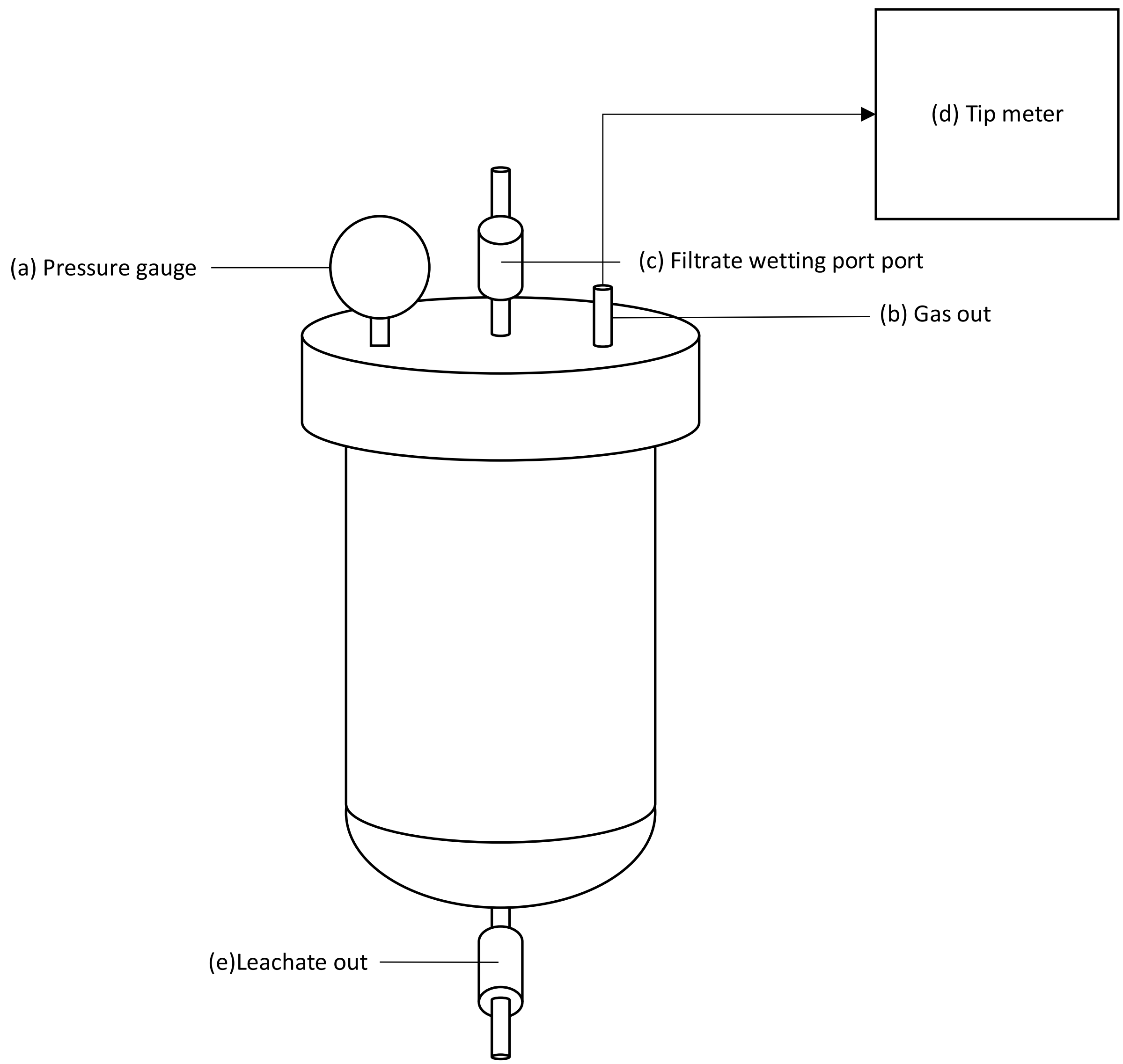


Figure 2: Diagram of a small-scale pilot dry anaerobic digester system

Three ports were drilled into the top flange to input a feeding port with a pressure gauge (Figure 2a), gas output line (Figure 2b), and filtrate wetting port (Figure 2c). The gas output line was connected to an accumulative gas flow meter (Wet Tip Gas Meter) filled with water and affixed with a counter (Figure 2d, Figure 3). Each tip corresponded to a volume of gas produced determined by calibrating the equipment. Tip meters were placed outside of the temperature-controlled room to minimize changes in calibration due to evaporation of water. A ball valve with an attached barb fitting was installed on the flange cap on the bottom of the digester for leachate output (Figure 2e). One meter of tubing was connected to the barb fitting on the bottom and the other end was connected to a barb fitting with attached ball valve. The pilots were placed in a temperature-controlled room (temperature measured daily 37°C±1) (Figure 4).

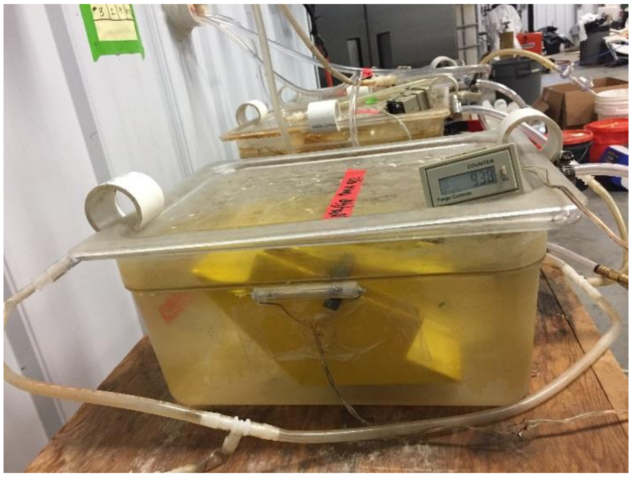


Figure 3: Tip meter connected to pilot digesters

Figure 4: Pilot set-up in temperature-controlled room

3.8.1 Pilot monitoring

Pilots were monitored each day during feedings. The time, number of tips, amount filtrate fed, air temperature and pressure was read and recorded. Leachate production was also monitored and emptied as necessary.

3.8.2 Feeding

Filtrate from the MSUSCAD was used to feed the pilots. Each pilot system was run on a 60-day feeding schedule that was tapered down every two weeks with the most leachate being fed during the two weeks. Manual feedings were scheduled 4 times per day at 8 am, 10 am, 2 pm, and 4 pm. If a scheduled feeding time was missed, the digester was fed at the next scheduled feeding with both the missed amount and the new feed amount required. On average, the pilots were scheduled to be wetted 12.5 mL per day (Table 4).

Table 4: Pilot feeding schedule

Week	**Volume (mL/day)**
1	20
2	20
3	16
4	16
5	10
6	10
7	4
8	4
Average	**12.5**

To feed the system, the required amount was drawn into a 60 mL syringe that was subsequently inserted into the barb fitting on the feed port. The ball valve to the feed port (Figure 2) was opened and the contents of the syringe were ejected into the digester. The ball valve was then closed and the syringe removed.

3.8.3 Leachate collection

During the course of the test, the ball valve attached to the digester was left open while the ball valve on the opposite end remained closed to allow the tubing to fill with leachate. Leachate was collected each time the outlet tubing was full by closing the ball valve connected to the outlet and opening the other. The volume of leachate out was recorded by pouring the leachate into a 50 mL graduated cylinder. The leachate pH was also measured and recorded.

3.8.4 Gas

Cumulative gas production was calculated using Equation 19.

$$G_C = T * C \tag{19}$$

G_C	cumulative gas production, mL
T	number of tips
C	calibration of tip meter, mL

Gas analysis was performed weekly to record CH_4, CO_2, and H_2S. To collect a sample for GC analysis a 5 mL SGE Analytical Science syringe was used. The syringe was connected to the gas sampling port on the gas output line before the tip meter (Figure 5). Once connected, the syringe was flushed by pulling and plunging slowly three times. Three mL of sample was then drawn into the syringe and the syringe was connected to the gas chromatograph.

Figure 5: Gas sampling port connected to the gas outlet tubing from the small-scale pilot digesters prior to the gas entering the tip meter

3.8.5 Digestate

The solid digestate was collected after the pilot systems were taken apart. TS, VS, and NREL tests were performed on the material. A sample was also sent to A&L Great Lakes Laboratories for CN testing.

3.8.6 Model Fitting

A simplified model was used to describe biogas production per kg initial VS over the course of the test. The Modified Gompertz equation (Equation 20), described by Donoso-Bravo and others (2010) and Lay, Li, & Noike (1997), was fit to the pilot data for both the zoo mix and ZMF samples to estimate the performance parameters (P, R_m, and $\boldsymbol{\lambda}$). Duplicate data sets were used for the fitting to determine the performance parameters.

$$M = P * \exp\left(-\exp\left(\frac{R_m * e}{P} * (\lambda - t) + 1\right)\right) \tag{20}$$

P	maximum biogas production, L biogas/kg initial VS
R_m	maximum biogas production rate, L biogas/kg initial VS/day
$\boldsymbol{\lambda}$	lag phase time, day
e	Euler's number
t	time, day

In order to determine if the parameters could be identified the scaled sensitivity coefficients (SSCs) were calculated with the method described by Beck & Arnold (1977), using initial parameter estimates based on the data. The SSCs are representative of the sensitivity of the model to the parameters. To calculate the SSCs for a model $\eta(x, t, \beta)$, where x and t are independent variables and β is the parameter vector, the i^{th} sensitivity coefficient was first calculated using Equation 21 and a forward difference approximation of the first derivative.

$$X_i = \frac{\partial \eta}{\partial \beta_i} \tag{21}$$

It is desirable to have the SSCs be large relative to η, the dependent variable, and uncorrelated with each other. To compare the sensitivity coefficients, the sensitivity coefficients were scaled to find the SSC using Equation 22.

$$X_i' = \beta \frac{\partial \eta}{\partial \beta_i} \tag{22}$$

The parameters were then estimated using nlinfit, a MATLAB nonlinear regression algorithm using ordinary least squares. From the MATLAB analysis, the root mean square error

(RMSE), standard error, relative error, residuals, and confidence intervals were determined. The RMSE determined the goodness-of-fit of the data to the model and should be low relative to the scale of the model. The most accurate parameters will have the lowest relative error and largest SSC. The 95% confidence intervals were determined for each parameter. The confidence bands were plotted along with the bootstrapping confidence and prediction bands. Bootstrapping was done with the Monte Carlo method of bootstrapping the residuals. Appendix D shows the MATLAB code used to fit the data to the model and perform the statistical analysis.

4. CHARACTERIZATION AND BIOCHEMICAL METHANE POTENTIAL

4.1 Characteristics of animal wastes

Waste samples from 28 different animals and enclosures were collected in June of 2016 for solids content characterization; results are summarized in Table 5. Results show high variability in samples, due to variation in animal types, sizes, bedding requirements, and sample content, among others. TS content of the wastes ranged from 172,354 to 915,028 mg per kg for red panda and bird mix, respectively, while VS content ranged from 146,359 to 858,617 mg per kg for veldt and bird holding, respectively. Notably, the bird samples were higher in TS and VS than other waste samples overall due to the high amount of bedding, with the bird mix having the highest TS at 915,028 mg per kg.

Table 5: Individual animal waste characterization

Sample	TS (mg/kg)	VS (mg/kg)	TS:VS	Moisture Content (%)
Aardvark	383,486	300,287	78%	61.7
Asian Horse[a]	411,719	303,860	74%	58.8
Barnyard	327,595	280,214	86%	67.2
Bird Breeding Pen[b]	749,369	726,463	97%	25.1
Bear	476,297	306,339	64%	52.4
Bird Holding[c]	880,300	858,617	98%	12.0
Bird Mix[d]	915,028	660,713	72%	8.5
Bison	232,897	166,506	71%	76.7
Bush Dog	417,496	200,557	48%	58.3
Camel	273,715	240,858	88%	72.6
Eland	414,745	260,038	63%	58.5
Free Flight Aviary[e]	647,869	632,836	98%	35.2
Giraffe	604,873	491,643	81%	39.5
Guanaco, Rhea, Deer Mix	494,779	404,056	82%	50.5
Great Ape	262,481	226,224	86%	73.8
Kangaroo	367,322	267,121	73%	63.3
Lion	438,353	321,817	73%	56.2
Amphibian Conservatory[f]	330,206	305,872	93%	67.0
Ostrich	465,576	307,053	66%	53.4
Red Panda	172,354	150,744	87%	82.8
Rhino	214,342	194,642	91%	78.6
Stork, Crane	850,055	801,961	94%	15.0
Tree Kangaroo	482,850	419,043	87%	51.7
Veldt[g]	339,276	146,359	43%	66.1
Warthog[h]	215,260	176,658	82%	78.5
Watering Hole[i]	459,696	303,850	66%	54.0
West Pampas[j]	413,363	373,634	90%	58.7
Zebra[k]	296,495	245,100	83%	70.4

a. Asian horse habitat contains Asian horse, vulture, camel, and deer.
b. Bird breeding pen types of bird vary during the year.
c. Bird holding types of birds vary during the year.
d. Bird mix includes flamingo, vulture, golden crown, spoonbill, and stork.
e. Free flight aviary contains a large variety of bird types.
f. Amphibian conservatory contains a large variety of amphibian types.
g. Veldt includes warthog and zebra and was collected along with bedding.
h. Warthog collected without bedding.
i. Watering hole contains flamingo, pelican, eland, and ostrich, among other bird types.
j. West pampas includes emu and flightless birds.
k. Zebra collected without bedding.

The total waste generation from animals at the Detroit Zoo is estimated to be 535,131 kg per year. Among these, the hoofstock animals produced approximately 508,824 kg wastes per year. The hoofstock mix was the largest portion and accounted for 95% (w/w) of the zoo animal wastes (Table 1, Figure 1a). Given this proportion, it was assumed that the hoofstock mix TS and VS content would be similar to the zoo mix. Table 6 gives the characteristics of animal wastes by category. TS and VS of the hoofstock mix (395,635 and 296,872 mg per kg) and zoo mix (397,555 and 295,159 mg per kg) were within the same range as each other. Animals wastes included in the hoofstock blend ranged from 214,342 to 604,873 mg per kg and 146,359 to 491,643 mg per kg for TS and VS, respectively. Variations in TS content were relative to the amount of bedding used, size of animal, number of animals in the habitat, and time of year the samples were collected. For example, the giraffe habitat had high TS at 604,873 mg per kg and a visual observation of the sample showed it was mostly bedding materials, while the rhino waste had relatively low TS at 214,342 mg per kg and appeared to contain very little hay and no bedding material. Visual appearance sample descriptions (i.e. primarily bedding, only manure, etc.) can be found in Appendix A.

The other 5% (w/w) of the waste include the bird mix at 11,123 kg per year (2.1% w/w), carnivore at 9,412 kg per year (1.8% w/w), and primate at 5,772 kg per year (1.1%) (Table 1, Figure 1b). Characterization of the wastes indicates that among zoo wastes, the bird mix has the highest TS and VS (693,219 and 650,055 mg per kg, respectively), followed by carnivore (431,385 and 296,196 mg per kg) and hoofstock mix (397,635 and 296,872 mg per kg); primate waste has the least TS and VS (255,789 and 215,909 mg per kg) (Table 6). Carnivore had much higher NH_3-N and COD concentrations than the other samples.

Table 6: Characteristics of animal wastes by category [a]

Sample	TS[a] (mg/kg)	VS[a] (mg/kg)	C:N[b]	pH	NH_3-N (mg/kg)	COD (mg/kg)
Carnivore	431,385±39,989	296,196±31,729	12±0	8.14	2,773	426,250
Primate	255,789±7,298	215,909±12,749	14±1	8.23	332	274,875
Hoofstock Mix	397,635±22,029	296,872±10,289	30±4	8.81	414	151,500
Bird Mix	693,219±158,271	650,055±116,185	27±6	6.96	265	152,500
Zoo Mix	397,555±22,786	295,159±19,696	26±9	8.64	401	262,000
Food Waste	327,084±72,973	274,839±27,144	14±5	4.17	540	343,000
ZMF	362,493±17,811	275,729±13,693	26±3	8.41	550	264,500

a. Data are average with standard deviation.
b. Data are average with standard error.

Elemental analysis was conducted to evaluate the CN ratio, NH_3, and COD. Data summarized in Table 6 present that bird and hoofstock mixtures have much higher CN ratios (27:1 and 30:1, respectively) than carnivore and primate (12:1 and 14:1, respectively). This indicates that carnivore and primate waste would likely need to be codigested with a high carbon source to increase the efficiency of digestion and reduce risk of ammonia inhibition (Yadvika et al., 2004). Additionally, the carnivore waste was very high in NH_3-N at 2,773 mg per kg indicating that if used as a mono-substrate, there could be a chance for ammonia toxicity (Crook & Gould, 2009).

Hoofstock and bird mixtures with high C:N ratio mixed with low CN ratio carnivore and primate wastes resulted in the CN ratio of zoo mix (26:1) being in the optimal CN ratio range (of 20:1 to 30:1) for anaerobic digestion (Crook & Gould, 2009). Considering available food waste, the zoo mix and food waste at a ratio of 90:10 was considered as another feed combination for anaerobic digestion (Figure 1d). The ZMF has slightly less TS and VS (362,493 and 275,729 mg per kg), and similar CN ratio (26:1) compared to the zoo mix (Table 6).

pH of the zoo samples was in the 8.00-9.00 range with the exception of the bird mix (6.96) (Table 6). Food waste had the lowest pH at 4.17. This indicates that food waste would not do well as a mono substrate for the stability of the system given that a low pH can hinder stability in the system and inhibit the production of methane.

4.2 BMP Test

Seven samples (carnivore, primate, hoofstock mix, bird mix, zoo mix, food waste, and ZMF) were tested along with the inoculum control in three separate BMP trials, with the exception of just two BMP trials run for primate. Each BMP sample was tested in triplicate with the exception of lost data points from laboratory error (breaking a serum bottle). The number of bottles (n) were averaged together to obtain results. Additional BMP data is located in Appendix B.

4.2.1 Pre and post-digestion characterization

Pre and post-digestion characterization was carried out to determine the anaerobic biodegradability of the samples. Table 7 contains the pre and post-digestion TS content, the TS reduction, and the percent reduction. Initial TS for all runs was approximately 10,000 mg per kg, except the control run of the seed at approximately 8,000 mg per kg. Each of the samples had a higher TS percent reduction than the seed. This was due to the higher amount of total solids, and that there were more easily degradable compounds in fresh feedstocks, opposed to previously digested material. The food waste sample achieved the highest percentage reduction (33%) followed by the zoo mix and ZMF samples (27% and 26%, respectively).

Table 7: Pre and post-digestion TS content in BMP bottles

Sample	Pre-digestion Average ± Std. Dev. (mg/L)	Post-digestion Average ± Std. Dev. (mg/L)	Reduction (mg/L)	Reduction (%)	n
Seed	7,927±738	6,258±561	1,669	21%	9
Carnivore	10,716±1,741	8,122±944	2,593	24%	9
Primate	10,786±1,694	8,094±937	2,691	25%	5
Hoofstock Mix	9,916±579	7,567±463	2,349	24%	8
Bird Mix	9,485±762	7,115±496	2,370	25%	9
Zoo Mix	9,838±967	7,141±564	2,698	27%	9
Food Waste	10,067±1,458	6,758±738	3,310	33%	9
ZMF	10,489±1,206	7,807±666	2,682	26%	8

Table 8 contains the pre and post-digestion VS content, the mass of VS reduced, and the percent reduction. Similar to TS reduction, food waste had the highest VS reduction percent (43%), while the hoofstock mix was the lowest.

Table 8: Pre and post-digestion VS content in BMP bottles

Sample	Pre-digestion Average ± Std. Dev. (mg/L)	Post-digestion Average ± Std. Dev. (mg/L)	Reduction (mg/L)	Reduction (%)	n
Seed	5,599±542	4,019±274	1,581	28%	9
Carnivore	7,717±437	5,037±250	2,680	35%	9
Primate	8,042±1,190	5,279±609	2,763	34%	5
Hoofstock Mix	7,154±972	4,943±765	2,211	31%	8
Bird Mix	7,044±824	4,761±368	2,283	32%	9
Zoo Mix	7,200±695	4,713±464	2,487	35%	9
Food Waste	7,594±411	4,315±733	3,279	43%	9
ZMF	7,669±543	5,112±596	2,557	33%	8

Table 9 summarizes the pre and post-digestion COD characteristics and the percent reduction. Again, food waste had the greatest percent reduction indicating it is the most readily biodegradable.

Table 9: Pre and post-digestion chemical oxygen demand in BMP bottles

Sample	Pre-digestion Average ± Std. Dev. (mg/L)	Post-digestion Average ± Std. Dev. (mg/L)	Reduction (mg/L)	Reduction (%)	n
Seed	10,122±1,780	7,033±472	3,089	31%	9
Carnivore	13,500±1,963	8,067±990	5,433	40%	9
Primate	13,500±1,671	8,580±428	4,920	36%	5
Hoofstock Mix	12,688±1,339	7,444±916	5,244	41%	8
Bird Mix	10,789±1,673	6,806±476	3,983	37%	9
Zoo Mix	11,383±1,698	7,350±775	4,033	35%	9
Food Waste	13,256±1,830	7,106±848	6,150	46%	9
ZMF	10,900±1,359	8,450±1,269	2,450	22%	8

Table 10 contains the pre and post-digestion ammonia-nitrogen in the BMP test and the percentage increase. The hoofstock mix had the highest increase in NH_3-N at 61%. Given that all of the post-digestion pH levels are above 7.00 and the pre and post-digestion values are below the level of ammonia toxicity (3,000 mg per L), the digestion process will not be inhibited due to ammonia concentration (Crook & Gould, 2009).

Table 10: Pre and post-digestion ammonia-nitrogen in BMP bottles

Sample	Pre-digestion Average ± Std. Dev. (mg/L)	Post-digestion Average ± Std. Dev. (mg/L)	Accumulated (mg/L)	Increase (%)	n
Seed	464±247	675±168	210	45%	9
Carnivore	493±305	772±175	278	56%	9
Primate	651±134	709±180	58	9%	5
Hoofstock Mix	473±231	759±246	286	61%	8
Bird Mix	475±300	609±153	134	28%	9
Zoo Mix	460±265	692±325	232	50%	9
Food Waste	504±272	638±230	134	27%	9
ZMF	505±275	768±238	263	52%	8

Figure 6 summarizes the percent reduction of TS, VS, and COD, and increase in NH_3-N.

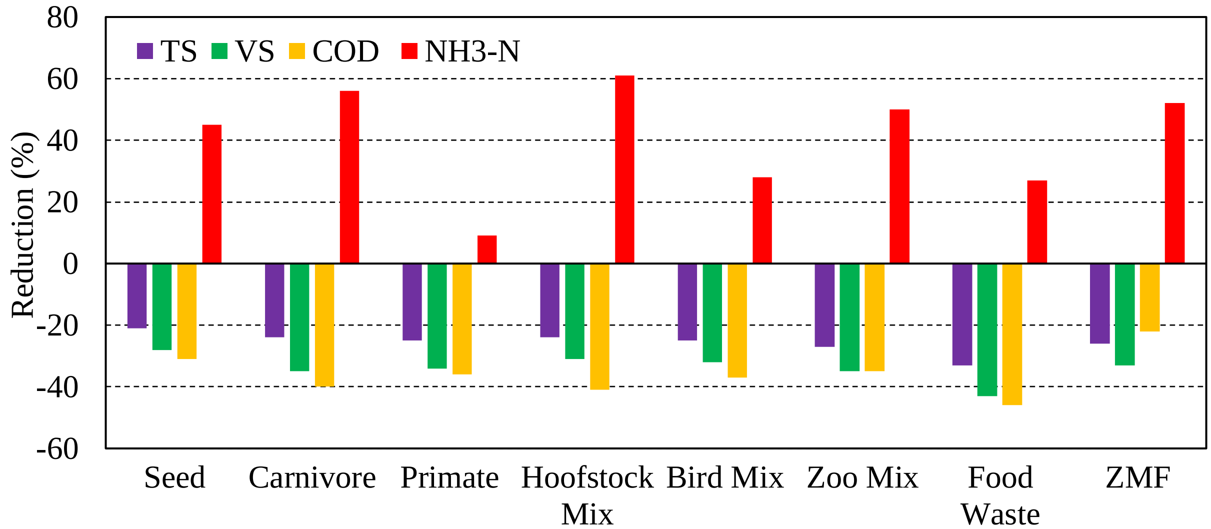


Figure 6: Reduction of TS, VS, COD, and increase NH_3-N during the BMP testing

Table 11 contains the pre and post-digestion pH characteristics and difference after digestion. All post-digestion samples pH were in the optimal range for anaerobic digestion indicating a stable environment during the BMP test (Liu et al., 2008).

Table 11: Pre and post-digestion pH in BMP bottles

Sample	Pre-digestion	Post-digestion	Difference	n
Seed	8.13	7.46	-0.66	9
Carnivore	7.99	7.32	-0.67	9
Primate	8.03	7.45	-0.57	5
Hoofstock Mix	8.02	7.32	-0.70	8
Bird Mix	8.04	7.46	-0.58	9
Zoo Mix	8.08	7.32	-0.76	9
Food Waste	7.79	7.33	-0.46	9
ZMF	8.17	7.28	-0.89	8

Average cumulative biogas production data from the BMP test show that during the 30 days test, carnivore, primate, hoofstock mix, bird mix, zoo mix, food waste, and ZMF generated 339, 438, 272, 275, 318, 472, and 343 mL biogas, respectively (Figure 7, Table 12). Food waste and primate had the highest cumulative biogas production (472 and 438 mL biogas) among all samples. As for reduction of TS, VS, COD, and NH_3-N, food waste had significantly higher TS, VS, COD reduction (33%, 43%, and 46%, respectively) than other samples, while hoofstock mix had highest NH_3-N accumulation (61%) among all samples (Figure 6).

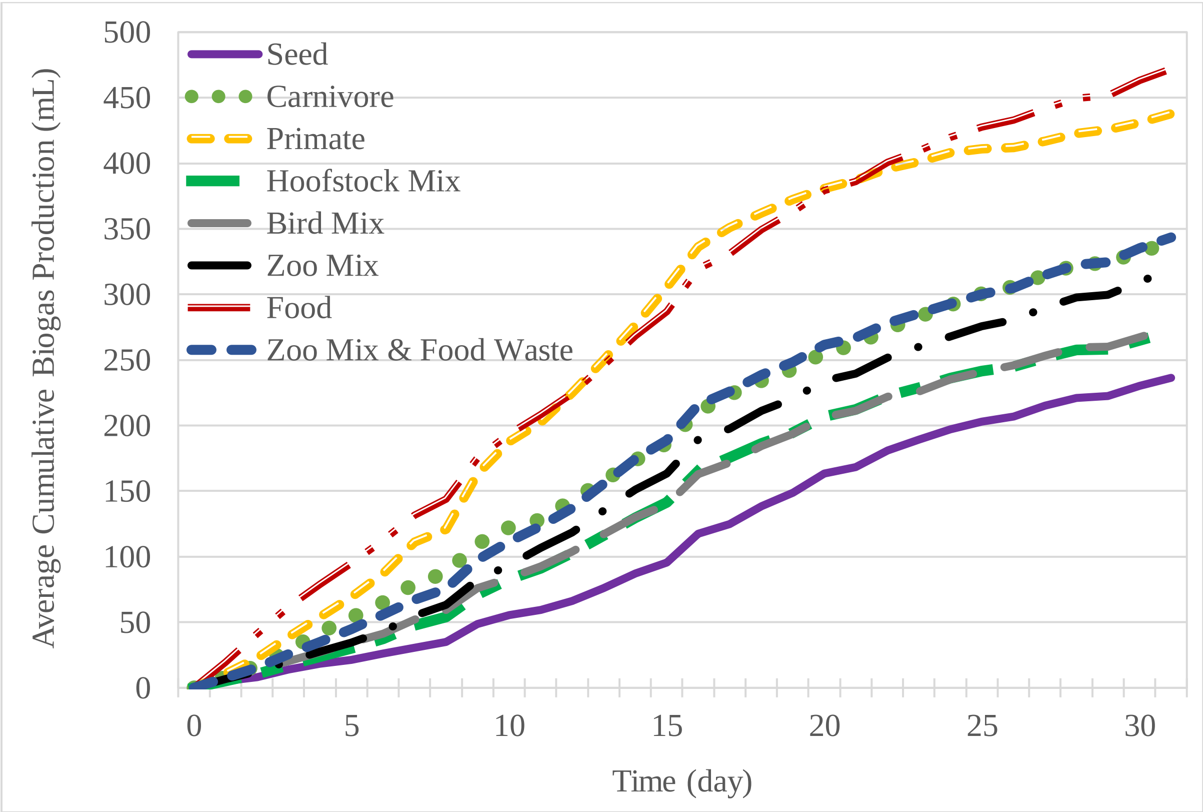


Figure 7: Average cumulative biogas production from BMP testing

Table 12: Total average cumulative biogas production from 30-day BMP test

Sample	**Average Cumulative Gas Production (mL biogas)**
Seed	236±79
Carnivore	339±14
Primate	438±63
Hoofstock Mix	272±25
Bird Mix	275±81
Zoo Mix	318±69
Food	472±25
ZMF	343±60

Biogas production and VS reduction were used to evaluate the digestion performance of individual samples and mixes. Primate and food waste again had highest BMP of 622 and 653 L biogas per kg initial VS, respectively, among all samples (Table 13). Even though carnivore and primate samples yielded the highest biogas production, their available quantities only make up 3% of the overall zoo mix. The hoofstock mix as the largest waste of the overall zoo mix had a BMP of 269 L biogas per kg initial VS. The BMP of the hoofstock mix is in the same range with other hoofstock animal BMP results (Kafle & Chen, 2016). Since hoofstock mix is the major composition of the zoo mix, BMP of the zoo mix (232 L biogas per kg initial VS) was not significantly different from the hoofstock mix (269 L biogas per kg initial VS) (Table 13).

Table 13: Biochemical methane potential of zoo wastes [a, b]

Sample	BMP (L biogas/ kg initial VS)	Average methane (%)	Maximum methane (%)	BMP_m (L methane/ kg initial VS)	(n)
Carnivore	501±22	56	68	280±41	9
Primate	622±42	60	70	374±3	5
Hoofstock Mix	269±44	61	71	164±36	8
Bird Mix	117±75	58	71	69±46	9
Zoo Mix	232±43	59	71	137±27	9
Food Waste	653±138	63	73	411±109	9
ZMF	302±40	60	71	183±33	8

a. All data are average with standard deviation.
b. The BMP values were corrected for the methane produced by the seed in the mixture.

In addition, the results of cumulative biogas and BMP clearly demonstrate that food waste addition improved digestion performance and increased biogas production. The mixture of 90% (w/w) zoo mix and 10% (w/w) food waste had a BMP of 302 L biogas per kg initial VS, which is 30% more than zoo mix alone (232 L biogas per kg initial VS). The samples all yielded similar average CH_4 percentages. Considering both BMP and available quantities of above tested samples, zoo mix and ZMF were selected to run mass and energy balance in the following section.

4.3 Theoretical Mass and Energy Balance and Carbon Footprint

The mass and energy balance was conducted to compare the digestion performance with zoo mix and ZMF (Table 14). Although the zoo digester is a high-solids dry digestion system, a completely stirred tank reactor (CSTR) was assumed as the digester for theoretical analysis given that the BMP data are representative of wet digestion systems. TS_{Feed} and retention time were set at 15% and 30 days, respectively. Using data from BMP (Table 13), the CH_4 production of zoo mix and ZMF were calculated to be 22 and 29 g per kg dry feed, respectively. Based on the amount of CH_4 generated and local environmental condition, the energy balance analysis concluded that with implementation of a CHP unit, net electricity outputs of zoo mix and ZMF were 0.09 and 0.12 kWh-e per kg dry feed, respectively, and corresponding net heat outputs were 0.01 and 0.07 kWh-e per kg dry feed. Due to the relatively low annual average temperature in Detroit (10°C), thermal energy requirements to heat the feed and maintain the digester temperature were considerably high. The energy generation efficiencies (net energy output per CH_4 energy × 100) were 29% and 42% for zoo mix and ZMF, respectively. Even though the energy generation efficiencies were relatively low, both feeds showed the positive efficiencies. Particularly, the addition of food waste is able to increase the gas production and improve the energy generation efficiency.

Based on the mass and energy balance data (Table 14), anaerobic digestion of 535,131 wet kg per year animal waste from DZS can produce 4,847 kg CH_4 per year, and corresponding net heat and electricity outputs are 1,034 kWh-e and 20,219 kWh-e, respectively. With addition of food wastes (ZMF ratio of 90:10), the CH_4 production, net heat, and electricity outputs were increased to 6,835 kg, 15,344 kWh-e, and 28,510 kWh-e per year, respectively. It has been reported that average electricity and heat demands of zoo are 553 kWh-e and 1,012 kWh-e per animal per year, respectively (Kusch, 2012). DZS has approximately 2,000 animals, and correspondingly requires 1,106,000 and 2,024,000 kWh-e per year of electricity and heat, respectively for the animal operations. The energy produced from anaerobic digestion of ZMF can contribute 1.4% of the total energy demand of the zoo animal operations.

Table 14: Theoretical mass and energy balance of anaerobic digestion of zoo wastes

	Zoo mix	ZMF
Mass balance		
Methane production (M, g/kg dry feed) [a]	22.47	29.06
Energy balance [b]		
Heat input (W_{heat}, kWh-e/kg dry feed) [c]	-0.20	-0.20
Electricity input ($W_{electricity}$, kWh-e/kg dry feed) [d]	-0.01	-0.01
Energy output as heat (E_{heat}, kWh-e/kg dry feed) [e]	0.21	0.27
Energy output as electricity ($E_{electricity}$, kWh-e/kg dry feed) [f]	0.10	0.13
Net energy output		
Net heat output (kWh-e/kg dry feed) [g]	0.01	0.07
Net electricity output (kWh-e/kg dry feed) [h]	0.09	0.12

a. Eq. 1 was used to calculate the methane production.
b. Negative numbers mean energy inputs, and positive numbers mean energy outputs.
c. Eq. 4 was used to calculate the heat input.
d. Eq. 5 was used to calculate the electricity input.
e. Eq. 2 was used to calculate the energy output as heat.
f. Eq. 3 was used to calculate the energy output as electricity.
g. The net heat output = $E_{heat} - W_{heat}$
h. The net electricity output = $E_{electricity} - W_{electricity}$

Even though the energy generation from anaerobic digestion only contributes a small portion to the total zoo energy demand, containing zoo wastes has a significant impact on reducing carbon footprint of the zoo (Figure 8). The total CO_2 emission of the zoo with landfill as the waste treatment was 1,450,000 kg CO_2-e per year with 53%, 25%, and 21% of the emission from electricity use, heat use, and waste treatment. With implementation of anaerobic digestion, the total CO_2 emission of the zoo was reduced to 1,210,000 kg CO_2-e per year with only 7% of the emission from the waste treatment, which are 16% lower than the emission with landfill (1,450,000 kg CO_2-e per year with 21% of the emission from the waste treatment), as well as lower than the emission with composting (1,270,000 CO_2-e per year with 10% of the emission from the waste treatment).

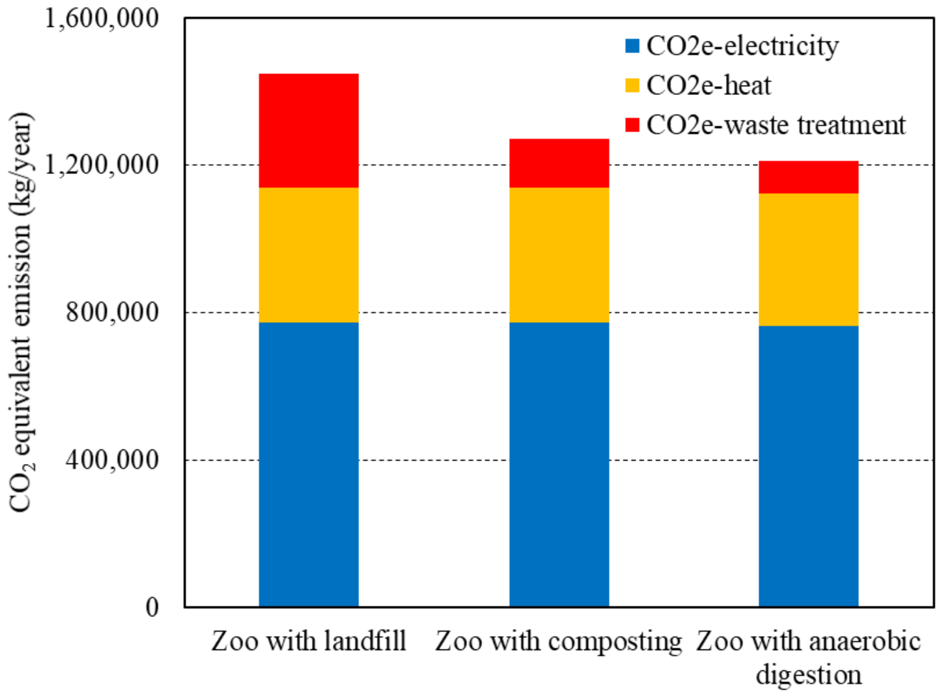


Figure 8: Carbon footprint of zoo with different waste treatment processes

5. PILOT TESTING AND FITTING A MODEL TO DATA FOR DETERMINATION OF DIGESTER PERFORMANCE PARAMETERS

5.1 Purpose

Given the limited information on anaerobic digestion of zoo organic wastes and dry digestion systems, it was necessary to test the anaerobic biodegradability. BMP testing, discussed in Chapter 4, is a standard parameter for which to compare different feedstocks with one another and is done in a batch, wet anaerobic digestion condition. Dry, batch, anaerobic digesters were constructed to allow for analysis of zoo organic wastes in dry anaerobic digestion conditions. By fitting a model to the pilot data, performance parameters can be determined and decisions can be made to improve operations to increase digestion and gas production in the commercial-scale system.

5.2 Results and discussion

The zoo mix and ZMF mixture, collected in October 2017, were tested in duplicate in batch anaerobic digesters. Neither mixture included carnivore nor primate wastes because they are not being utilized for the commercial-scale system. This is due to concerns with parasites in the effluent, and their relatively small contribution (less than 3% of total, Table 1) to the overall waste generation.

5.2.1 Characterization

Pre and post-digestion characteristics were analyzed to determine the biodegradability of the material in the pilot digester. Table 15 shows the pre and post digestion TS characterization mass reduced, and the percent reduction of TS. The zoo mix samples had a higher total solids content (569,776 mg per kg) than the ZMF (420,690 mg per kg), and a higher average percent reduction (48% and 41% for zoo mix and ZMF, respectively) in TS over all, which corresponds to the higher gas production seen in those samples.

Table 15: Pre and post-digestion total solids content in pilot digesters

Sample	Pre-digestion (mg/kg)	Post-digestion (mg/kg)	Reduction (mg/kg)	Reduction (%)
Zoo Mix (1)	569,776	320,128	276,648	44
Zoo Mix (2)	569,776	273,399	323,377	52
ZMF (1)	420,690	222,629	198,061	47
ZMF (2)	420,690	278,240	142,450	34

Table 16 shows the pre and post-digestion VS characteristics for the pilot digesters. Like the TS, the zoo mix pilots saw the greatest reduction in volatile solids with zoo mix ranging from 50 to 55% and ZMF ranging from 32 to 48%. As volatile solids are converted into volatile fatty acids and then used by methanogens to produce gas, the higher VS percent reduction corresponds with a higher level of gas production and enhanced digestion.

Table 16: Pre and post-digestion volatile solids in pilot digesters

Sample	Pre-digestion (mg/kg)	Post-digestion (mg/kg)	Reduction (mg/kg)	Reduction (%)
Zoo Mix (1)	295,864	147,847	148,017	50
Zoo Mix (2)	295,864	133,749	162,115	55
ZMF (1)	260,865	136,686	124,179	48
ZMF (2)	260,865	177,869	82,996	32

Table 17 shows the CN ratios for pre and post-digestion. The ZMF sample had a lower CN ratio than the zoo mix, but both samples are in or close to the optimal range for digestion.

Table 17: Pre and post-digestion carbon-nitrogen ratio

Sample	Pre-digestion
Zoo Mix (1)	31.2
Zoo Mix (2)	31.2
ZMF (1)	27.1
ZMF (2)	27.1

5.2.2 Gas Production

The leachate began discharge from the pilot much earlier in the ZMF pilots with leachate production beginning around day 15, as opposed to day 20 for the zoo mix samples. Table 18 shows the cumulative volume of leachate collected from each of the pilots with 158.3, 99.6, 235.6, and 260.1 mL of leachate produced during the test for the zoo mix (1), zoo mix (2), ZMF (1), and ZMF (2), respectively. On average, the ZMF pilots leached nearly 119 mL more than the zoo mix sample over the course of the test.

Table 18: Pilot digester leachate volume and pH

Sample	Cumulative Volume (mL)
Zoo Mix (1)	158.3
Zoo Mix (2)	99.6
Average Zoo Mix	**129.0**
ZMF (1)	235.6
ZMF (2)	260.1
Average ZMF	**247.9**

Figure 9 shows the cumulative biogas production from each of the pilot digesters. The zoo mix samples showed a higher cumulative gas production than the food waste, and the duplicate points were much closer to each other. Given the trend found in the BMP results where the food waste increased production, it is clear that there was an inhibitory effect of the food waste in the pilot digesters, which was not seen in the BMP results. A t-test of the total cumulative gas production showed that the zoo mix and ZMF results were significantly different.

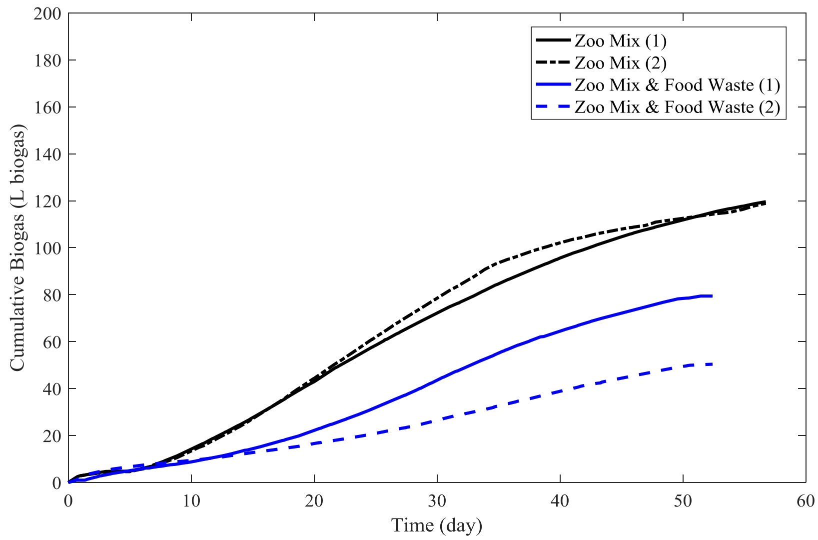


Figure 9: Cumulative biogas production from each pilot digester

The conditions of the digesters were measured by analyzing the leachate production pH over the course of the test and determining the volume leaching from the cells. Figure 10 shows the pH content over time for each sample collected. The data indicates that the ZMF digesters have a clearly lower pH than the zoo mix digesters. By day 25, the pH reached above 7.00, but it is clear there was an inhibitory effect of the pH on the gas production, and the microbes did not recover. One study performed on the influence of pH and moisture content in high-solids digestion found that in a digester with a pH lower than 6.1 or higher than 8.3, failure can occur (Lay et al., 1997). For the ZMF samples, both digesters were at or near (6.04 and 6.38 for ZMF 1 and 2, respectively) this critical threshold, and do not reach above 8.00 until between days 30 and 40. While this shows there may be some recovery of the stability, the inhibitory effect of the low beginning pH still produced a lower gas production overall.

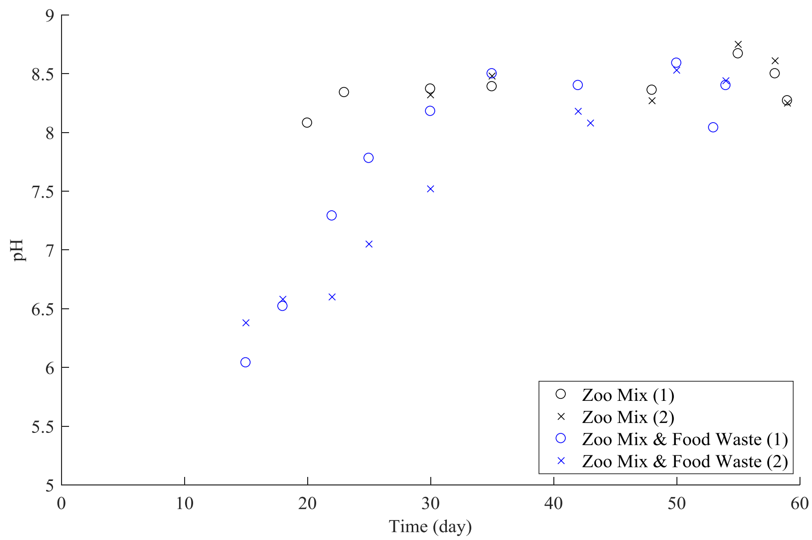


Figure 10: Leachate pH as collected in 60 day period

This indicates that the added filtrate may not have the buffering capacity to maintain the pH in the system with the addition of low pH food waste. More leachate will need to be recirculated in the beginning of the test or a buffer will need to be added in order to increase the pH and maintain the system stability. A study that looked at the effect of pH on high solids anaerobic digestion of food waste set up four samples (untreated, pH 7, pH 8, and pH 9) concluded that pH 8 reached the maximum methane yield, 7.57 times higher than the untreated sample (Yang et al., 2015). Figure 10 shows that after day 30, where there was an increase in the pH for ZMF (1), there is also an increase in the rate of gas production (Figure 11). ZMF (2) did not reach above 8.00 until day 40, and the rate of production remained relatively constant until the end of the test. This can also be seen in the cumulative gas production (Figure 9), where the difference in cumulative production for each sample diverges more drastically after that point and gas production does not recover in ZMF 2.

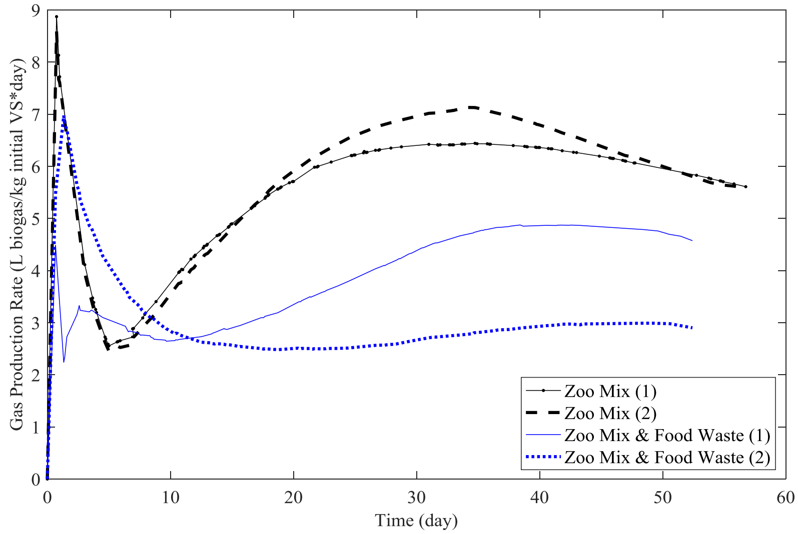


Figure 11: Rate of gas production during pilot testing

Table 19 summarizes the total biogas per initial VS produced on day 30, and on day 50. The results show that on day 30, there was 192, 209, 131, and 80 L biogas per kg VS produced for zoo mix 1, zoo mix 2, ZMF 1, and ZMF 2, respectively. In all pilots, aside from zoo mix and food waste 1, the majority of gas production occurs in the first 30 days. Due to the pH and rate of gas production, ZMF 1 produces the majority of its gas after day 30. Given that on average, the majority of gas production occurs in the first 30 days of the test, in scale-up to a commercial system it may not be worth running the batches for longer than 30 days, but this operational parameter would also be dependent on the goals of the project.

Table 19: Biogas production at day 30 and day 50 of pilot test

Sample	Day 30 Biogas Production (L biogas/ kg initial VS)	Day 50 Biogas Production (L biogas/ kg initial VS)	Increase (%)
Zoo Mix (1)	192	226	18
Zoo Mix (2)	209	260	24
Average	**201**	**243**	**21**
ZMF (1)	131	210	60
ZMF (2)	80	96	20
Average	**106**	**153**	**40**

5.2.3 Gas chromatography analysis

Gas chromatography was performed on the samples typically once per week from each of the digesters. The maximum and average CH_4 values are given in Figure 12. In both the zoo mix and the ZMF pilots, the digesters reached around 50% methane around day 13 and remained relatively constant (45-55%) until the end of the test.

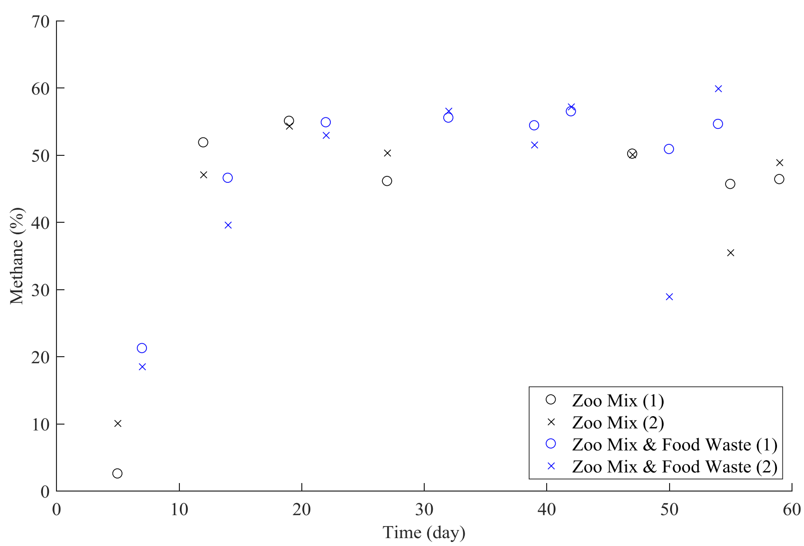
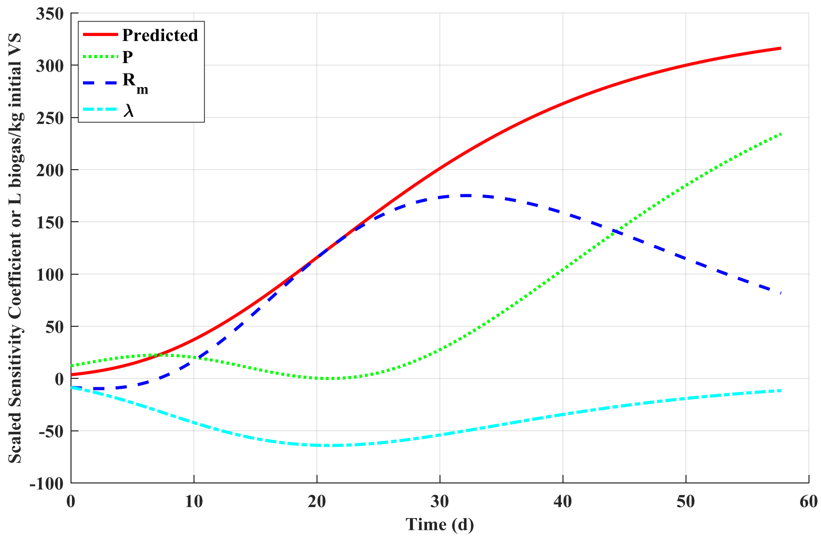


Figure 12: Pilot methane content from gas chromatography analysis

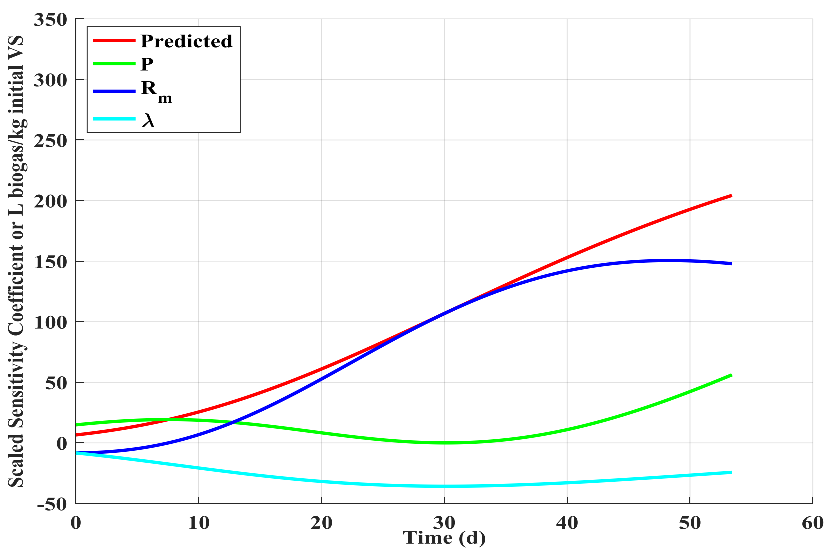
5.3 Fitting the pilot data to a simplified anaerobic digestion model

A simplified practical model was fit to the in order to determine performance parameters from the zoo mix and ZMF pilot data. Using the Modified Gompertz equation (Equation 20), described by Donoso-Bravo and others (2010) and Lay, Li, & Noike (1997), the model was fitted to determine the following parameters: maximum biogas production (P, L biogas/kg initial VS), maximum rate of biogas production (R_m, L/kg initial VS*day), and the lag phase time (λ, day) (Donoso-Bravo et al., 2010; Lay et al., 1997).

To determine if the parameters could be adequately identified, the scaled sensitivity coefficients (SSC) were calculated using initial parameter estimates and the averaged pilot data for the zoo mix and ZMF samples. A large (>10% of the total scale) and uncorrelated SSC will provide the most accurate estimate results. Figures 13a and 13b show the SSCs for the zoo mix and ZMF models, respectively. Since the SSCs were large, and uncorrelated, the parameters could be individually identified. The zoo mix SSCs show that parameter P is the largest relative to the scale and will have the lowest relative error. It is also clear that it takes a longer experimental time to estimate P, likely more than 30 days, whereas R_m and λ can be estimated after a shorter time. The ZMF SSCs show that parameter R_m is the largest and will have the lowest relative error. Parameter P (Figure 13b) is small until after day 40, so the experiment needs at least 40 days to accurately estimate P.



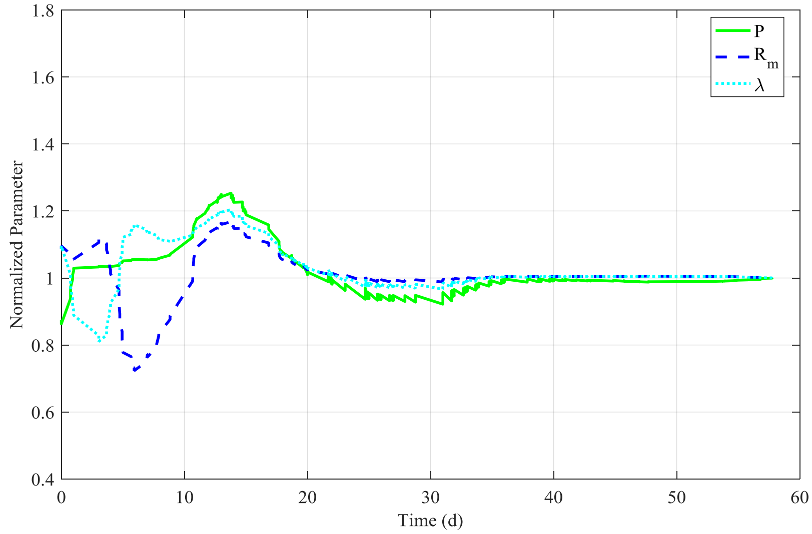
(a) Zoo Mix



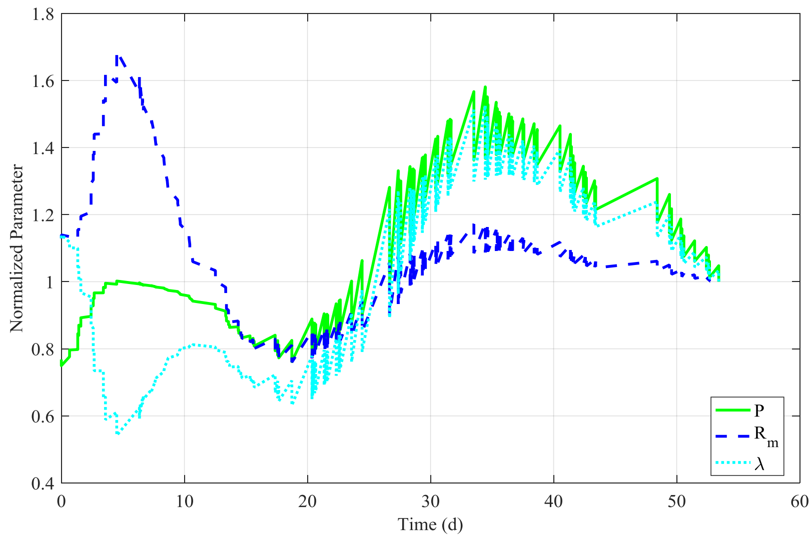
(b) ZMF

Figure 13: Scaled sensitivity coefficients

Sequential analysis determines the parameter response as more data are introduced over time. Sequential estimation is important to determine the amount of time the experiment needs to run for accurate determination of the parameters. Figures 14a and 14b show the sequential analysis of the zoo mix and ZMF models, respectively. It is clear from the sequential plots that the parameters are more accurately estimated for the zoo mix and variation is decreased as new data are introduced over time. The zoo mix sequential parameters converge around day 20, so future experiments will need at least 20 days for accurate parameter estimation, and more than 35 days will yield the most accurate results. This validates the preliminary analysis of the SSC plots. The ZMF SSCs take longer to converge. While some convergence can be seen after day 50, the ZMF samples may need additional time to more accurately estimate the parameters. This is likely due to the divergence of the two sets of data and will be more accurately estimated with additional data points.



(a) Zoo Mix



(b) ZMF

Figure 14: Normalized sequential parameter

Table 20 provides the parameter estimation and results of the statistical analysis. In comparing the model fitting, the zoo mix data has a better fit than the ZMF data. This is due to the higher variance in the ZMF data. The relative errors of the parameters for the zoo mix, 0.56, 0.75, 1.68% for parameters P, R_m, and $\boldsymbol{\lambda}$, respectively, were much lower than those for the ZMF, 11.4, 3.8, and 12.6, for P, R_m, and $\boldsymbol{\lambda}$, respectively. It is expected, given the trend of the ZMF data, that the lag time would be most accurately estimated, given that the variance increases as the time increases, and the lag time concerns only the beginning of the digestion time. The zoo mix has a low RMSE (<5% of total scale), indicating a good fit of the model to the data. The ZMF also has a low RMSE relative to the scale (<10% of total scale).

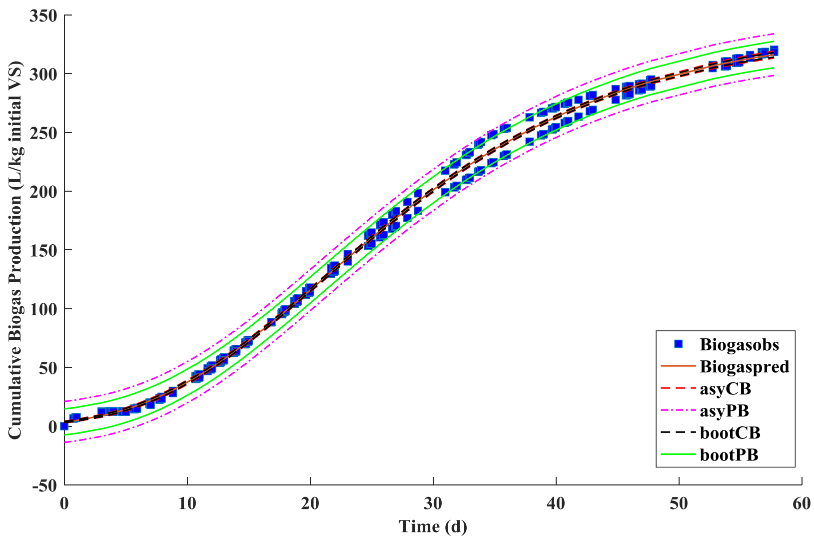
The parameter estimates show a higher maximum gas production (P) and maximum gas production rate (R_m) in the zoo mix, which was also indicated in the cumulative biogas results. The lag phase for both systems was similar at 7.1 and 7.5 days for zoo mix and ZMF, respectively.

Table 20: Parameter estimation and result of statistical analysis

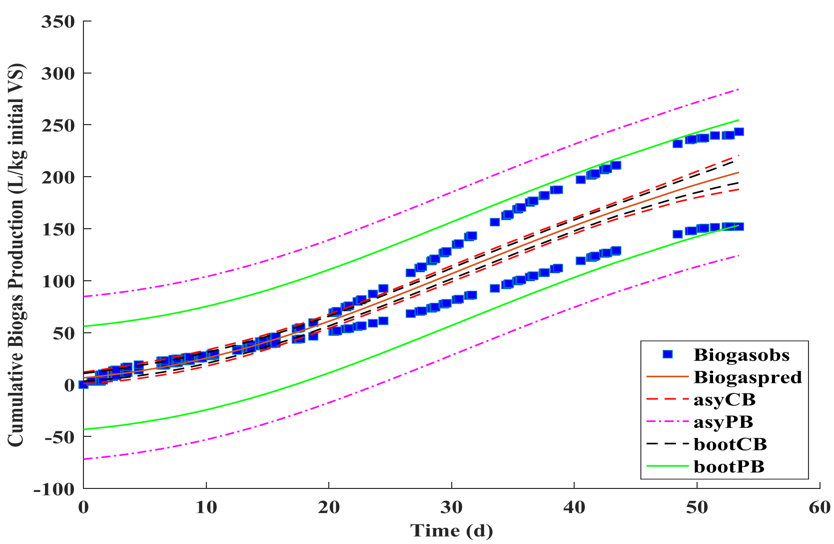
Sample	Parameter	Estimate	Relative Error (%)	95% Confidence Intervals	Mean of Residuals	RMSE	Maximum Correlation Coefficient
Zoo Mix	P	339.6	0.56	(335.8, 343.3)	-0.03	5.61	0.84
	R_m	9.0	0.75	(8.9, 9.1)			
	$\boldsymbol{\lambda}$	7.1	1.68	(6.9,7.4)			
ZMF	P	291.8	11.4	(226.1,357.5)	0.14	25.09	0.79
	R_m	4.8	3.8	(4.4, 5.1)			
	$\boldsymbol{\lambda}$	7.5	12.6	(5.7, 9.4)			

Further analysis of the residuals shows a signature or serial correlation in the results. This non-random pattern can indicate that there may be some variable missing from the model or there is a missing interaction between terms already in the model. Another possibility is that it is necessary to use different analysis technique that accounts for the serial correlation in the residuals.

Figures 20a and 20b show the observed data and model with the asymptotic confidence and prediction bands, and the bootstrapping confidence and prediction bands. The bootstrapping bands provide a slightly narrower range than the asymptotic bands. The zoo mix shows very narrow banding, with much of the data fitting within the bootstrapping and asymptotic confidence bands, indicating a good fit. The ZMF show much wider prediction bands, with only some of the data fitting within the confidence bands, indicating a worse fit.



(a) Zoo Mix



(b) ZMF

Figure 15: Model plotted with confidence and prediction bands

5.4 Scale-up and future considerations

Using this model in scale-up for a commercial system could improve design parameters and operational conditions. It is necessary to further the research under several operating conditions to develop more robust parameter estimates. The operating conditions that can be varied include leachate recirculation rate, temperature, in addition to varying the feedstocks used. Varying the leachate recirculation cycle will likely result in different parameter estimates for the zoo blend & food waste samples as it will influence the pH of the system. Additionally, future work may consider a more complex model that accounts for more of the variables in the system.

In considering the design of a high-solids anaerobic digester at a zoo, this model could help to determine key design aspects. The maximum biogas production could help in determining the generator size and amount of material needed for desired gas production. The maximum biogas production rate could help in sizing the gas storage or gas bladder and help in estimating maximum generator runtime.

6. OVERALL CONCLUSIONS AND RECOMMENDATIONS

6.1 Waste characterization and biochemical methane potential testing

The TS and VS content of the individual wastes and waste mixes were variable, with the bird samples having the highest TS content. Animal habitat bedding appears to be the primary driver in differences in solids and moisture content, with habitats using more bedding have higher solids content and lower moisture levels. Given that the zoo uses more bedding in the winter months and with new animal births, these results will likely vary throughout the year and additional data points will likely show this variance.

The BMP test of zoo wastes and waste mixes showed that all zoo wastes are anaerobically biodegradable. The BMP results for the hoofstock mixture (269±44 L biogas per kg initial VS), and the zoo mixture (232±43 L biogas per kg initial VS), were similar, given that the zoo mixture contained 95% hoofstock mixture. Published data for domestic livestock showed biogas production were in the range of 222 to 584 L biogas per kg VS. Carnivore and primate wastes had a much higher BMP, however they account for less than 3% of the total waste production from the zoo. This is likely because the samples contained more readily digestible material with higher energy content than samples containing lower energy content such as bedding and hay.

Mixing 10% of food waste with the zoo mix led to 30% increase on biogas production. Food waste achieved the highest TS, VS, and COD reduction, indicating that it is the most anaerobically biodegradable sample. Given solely the results of the BMP test, food waste will improve biogas production and increase the capacity for renewable energy generation and greenhouse gas reduction.

Mass and energy balances indicate that biogas from the anaerobic digestion of zoo wastes at the Detroit Zoo only replaces a small amount of fossil-based energy, though, the carbon footprint analysis indicated that 16% reduction of CO_2 emission was achieved. The results concluded that anaerobic digestion is an appropriate solution to manage zoo wastes, significantly reduce carbon footprint of the zoo, and generate renewable energy.

6.2 Pilot data and model fitting

6.2.1 Physical results

Pilot testing was able to achieve cumulative biogas production, on average, of 244 and 153 L biogas per kg initial VS for zoo mix and ZMF samples, on day 50. The zoo mix samples showed the highest TS and VS percent reduction. However, the results contradicted the BMP results, as the zoo mix yielded 90 L biogas per kg VS more than the ZMF in the pilot testing at day 50.

The inhibition of gas production in the ZMF pilots was shown in the low pH of the system, with the pH remaining between 6.00 and 7.78 in the ZMF pilots during the first 30 days, while the zoo mix pH was between 8.08 and 8.37 in the zoo mix. This indicates that it is necessary to increase the buffering capacity of the system in the beginning of the digestion process to maintain the pH in the acceptable range for optimal digestion. This could be done by increasing the amount of leachate sprayed onto the system or controlling the leachate pH by adding a buffering solution. Another possibility is to add a system to anaerobically digest the leachate in order to stabilize the pH through a microbial process. The effect of the pH was not seen in the BMP results due to the amount of filtrate in the system (20% filtrate in the bottle) that provided the buffering capacity to accommodate the low pH of the food waste.

Zoo mix (1) and zoo mix (2) rate of gas production decreased after days 35 and 36, respectively, indicating that a commercial-scale retention time of over 35 days will reach the maximum gas production. The ZMF samples saw their rates of production later in the time with ZMF (1) and zoo mix and food waste (2) reaching their maximum production rates after 43 and 49 days, respectively. The lag in achieving the maximum biogas production rate was the length of time it took for the pH to stabilize.

6.2.2 Modeling

The model developed from the Modified Gompertz equation (Equation 20) was able to predict performance parameters in a commercial scale digester, given the fit of the pilot data. The zoo mix fit the model better than the ZMF data. The SSCs of the parameters were determined to be large an uncorrelated, which indicated that the parameters could be estimated with low relative error. These results were confirmed by the low relative errors of the parameters (Table 20), which indicates a good parameter estimation. Results of sequential analysis indicates that the parameters could be estimated after day 20 for zoo mix and after day 50 for zoo mix and food waste. Future testing could shorten the length of the test and still accurately estimate model parameters. Statistical analysis shows that there is a signature in the residuals, which does not meet the standard statistical assumptions required to run ordinary least squares. This means either that a parameter apparent in the biology of the digestion is not being accounted for in the model or that a different residual analysis may be necessary to account for the serial correlation.

The model can be applied to commercial-scale design and operations. Given a known amount of zoological organic waste and using the characterization provided in this study, biogas production per kg initial VS can be estimated at a given time. This can aid in digester design to determine sizing for both the generator and chamber. The lag phase time can be used to assess system performance and determine when gas production will ramp up. The maximum gas production rate can be used to determine design parameters for the gas storage tank or bladder, in addition to generator size and runtime. Additionally, given the BMP finding that the zoo mix will behave similarly to other hoofstock animals, the model could be broadly applied to zoos with a similar percentage of hoofstock animals, regardless of the specific animal species percentage, to aid in digester design.

6.3 Future work

Given the limited data available on anaerobic digestion of zoological organic waste, many future design considerations could improve the knowledge gap that currently exists. BMP testing could be performed on individual animal wastes to provide data that are more robust and more accurately define the biogas potential ranges at zoos. Individual animal waste feedstocks could also be analyzed for other parameters such as pH, COD, NH_3-N, and CN ratio to continue to improve the research. There is currently a concern that primate and carnivore wastes may continue to harbor transmittable pathogens post-digestion and composting. Given the high BMP results from both of these substrates, future data should look at pathogen reduction in both anaerobic digestion and composting. This could be especially useful in zoos that have a high percentage of primate and carnivore animals.

Given the simplicity of the model, there are many improvements that could be made, and future work should look at comparing other models in addition to the Modified Gompertz Equation, such as those described by Donoso-Bravo and others (2010), or other more complex models. Additional research on pilot digestion could enhance the robustness of the model. Potential improvements could be to test additional zoo feedstocks (primate, carnivore, hoofstock, bird, varying food waste percentages, etc.) to provide a range of parameter values for zoos to more accurately apply the model to various zoo conditions. The leachate recirculation schedules can also be tested to optimize operations and improve digestion of the ZMF sample. Additionally, pilot data and the simplified model should be used for comparison against commercial-scale data to determine validity of the model.

APPENDICES

Appendix A: Visual description of zoo samples

This appendix gives information on the visual description of collected zoo samples.

Table 21: Visual description of zoo samples

Animal Type	Description
Aardvark	Mix of leaves, bedding, manure
Stork, Crane	Primarily bedding, hay, pellets
Kangaroo	Mix of bedding, hay, manure
Tree Kangaroo	Primarily bedding, leaves, twigs
Bison	Only manure
Rhino	Primarily manure, some hay
Camel	Primarily manure, some hay
Amphibian Conservatory	Primarily leaves, tree, dirt
Red Panda	Small amount of bedding
Great Ape	Only manure
Zebra	Only manure
Giraffe 2	Lots of bedding, leaves
Asian Horse	Mix of hay, variety of manure
Watering Hole	Primarily sticks, bedding, leaves
Veldt	Mix of hay, variety of manure
Lion	Only manure
Bush Dog	Only manure
Barnyard 2	Mix of hay, variety of manure
Eland	Primarily manure, some sticks, leaves
Guanaco, Rhea, Deer Mix	Mix of manure, sticks, hay, bedding
Free Flight Aviary	Primarily bedding
West Pampas	Primarily bedding
Bird Breeding Pen	Primarily bedding, hay, pellets
Ostrich	Primarily hay
Bird Holding	Primarily bedding, hay
Bird Mix 1	Primarily bedding, hay
Bird Mix 2	Primarily bedding, leaves, hay
Bear	Only manure
Warthog	Only manure

Appendix B: Additional BMP data

This appendix provides additional BMP data (raw, pre, and post-digestion analyses) for individual triplicate samples.

A1. Round 1 BMP

Table 22: BMP round 1 raw characterization

Sample	TS (mg/kg)	VS (mg/kg)	TS (%)	VS (%)	TS:VS
Filtrate	35,025	24,278	3.5	2.4	69%
Carnivore	459,786	288,187	46.0	28.8	63%
Primate	250,628	206,894	25.1	20.7	83%
Hoofstock Mix	413,212	302,812	41.3	30.3	73%
Bird Mix	805,134	717,134	80.5	71.7	89%
Zoo Mix	421,279	317,902	42.1	31.8	75%
Food Waste	275,485	259,168	27.5	25.9	94%
ZMF	375,088	283,635	37.5	28.4	76%

Table 23: Round 1 BMP pre-digestion data

Sample	pH	TS (mg/L)	VS (mg/L)	COD (mg/L)	Ammonia (mg/L)
Seed 1	8.22	7,132	5,020	10,850	675
Seed 2	8.29	7,263	5,122	7,750	685
Seed 3	8.32	6,582	4,685	6,900	666
Carnivore 1	8.19	10,352	7,362	11,800	735
Carnivore 2	8.23	9,937	7,188	12,700	753
Carnivore 3	8.22	10,130	7,265	11,500	801
Primate 1					
Primate 2	8.2	10,170	7,520	11,650	818
Primate 3	7.99	9,657	7,432	11,800	768
Hoofstock Mix 1	8.21	9,817	7,083	11,000	680
Hoofstock Mix 2	8.23	9,678	6,965	11,550	686
Hoofstock Mix 3					
Bird Mix 1	8.25	9,325	7,058	8,900	683
Bird Mix 2	8.15	8,645	6,488	8,500	673
Bird Mix 3	8.3	8,165	5,907	9,200	692
Zoo Mix 1	8.19	9,625	7,110	9,750	690
Zoo Mix 2	8.24	9,657	6,947	9,000	681
Zoo Mix 3	8.21	9,452	6,882	9,500	669
Food Waste 1	7.9	9,435	7,213	11,050	738
Food Waste 2	7.91	9,167	6,935	10,250	787
Food Waste 3	8.05	9,552	7,223	12,000	758
ZMF 1	8.27	10,002	7,313	9,850	708
ZMF 2	8.29	10,178	7,445	10,050	688
ZMF 3	8.28	9,697	7,080	8,850	691

Table 24: Round 1 BMP post-digestion data

Sample	pH	TS (mg/L)	VS (mg/L)	COD (mg/L)	Ammonia (mg/L)
Seed 1	7.58	6,082	4,140	6,800	584
Seed 2	7.59	5,945	3,915	6,600	560
Seed 3	7.62	6,055	3,988	6,950	520
Carnivore 1	7.33	8,153	5,065	8,900	591
Carnivore 2	7.40	7,952	4,972	9,100	614
Carnivore 3	7.40	8,178	5,025	9,300	587
Primate 1					
Primate 2	7.34	7,112	4,492	8,900	573
Primate 3	7.28	7,560	4,780	8,450	461
Hoofstock Mix 1	7.25	7,765	5,347	7,900	553
Hoofstock Mix 2	7.33	6,918	4,625	7,700	552
Hoofstock Mix 3					
Bird Mix 1	7.45	7,442	5,222	7,000	552
Bird Mix 2	7.43	6,963	4,810	7,300	542
Bird Mix 3	7.40	7,000	4,700	6,350	535
Zoo Mix 1	7.25	7,147	4,828	7,800	533
Zoo Mix 2	7.28	7,105	4,887	8,500	550
Zoo Mix 3	7.29	6,302	4,138	7,450	535
Food Waste 1	7.29	5,678	3,583	8,700	561
Food Waste 2	7.32	5,962	3,783	6,500	553
Food Waste 3	7.31	8,680	6,060	7,050	573
ZMF 1	7.21	8,680	6,060	8,650	556
ZMF 2	7.27	7,840	5,192	7,950	545
ZMF 3	7.32	7,822	5,315	9,500	561

Table 25: Round 1 BMP gas composition from weekly gas chromatography analysis

Sample	Average Methane (%)	Max Methane (%)	Average CO_2 (%)	Max CO_2 (%)	Average H_2S (%)	Max H_2S (%)
Carnivore	48	58	20	23	644	792
Primate	61	68	23	26	466	690
Hoofstock Mix	64	69	17	25	162	231
Bird Mix	58	68	14	17	160	199
Zoo Mix	60	67	15	20	163	248
Food Waste	67	71	23	24	455	729
ZMF	63	68	20	24	214	254

A2. Round 2 BMP

Table 26: BMP round 2 raw characterization

Sample	TS (mg/kg)	VS (mg/kg)	TS (%)	VS (%)	TS:VS
Filtrate	41,709	28,204	4.2	2.8	68%
Carnivore	448,715	331,162	44.9	33.1	74%
Primate	260,949	224,924	26.1	22.5	86%
Hoofstock Mix	413,212	302,812	41.3	30.3	73%
Bird Mix	805,134	717,134	80.5	71.7	89%
Zoo Mix	395,546	283,832	39.6	28.4	72%
Food Waste	275,485	259,168	27.5	25.9	94%
ZMF	375,088	283,635	37.5	28.4	76%

Table 27: Round 2 BMP pre-digestion data

Sample	pH	TS (mg/L)	VS (mg/L)	COD (mg/L)	Ammonia (mg/L)
Seed 1	8.05	8,430	5,741	10,650	571
Seed 2	8.13	8,289	5,651	10,100	575
Seed 3	8.07	8,346	5,727	10,050	585
Carnivore 1	7.80	11,739	8,430	16,000	626
Carnivore 2	7.96	11,059	7,554	14,100	613
Carnivore 3	7.90	11,180	7,693	14,450	606
Primate 1	7.83	12,173	9,095	14,100	545
Primate 2	7.79	9,405	6,685	14,700	521
Primate 3	8.33	12,525	9,480	15,250	606
Hoofstock Mix 1	8.15	11,350	8,325	13,600	610
Hoofstock Mix 2	8.07	10,908	8,105	12,200	588
Hoofstock Mix 3	7.93	11,315	8,287	12,400	625
Bird Mix 1	8.08	10,308	7,630	11,750	687
Bird Mix 2	7.92	9,627	7,078	11,850	620
Bird Mix 3	7.83	8,845	6,613	11,150	687
Zoo Mix 1	8.19	11,108	7,982	12,800	599
Zoo Mix 2	8.00	11,830	8,470	12,550	598
Zoo Mix 3	8.14	10,343	7,575	12,900	568
Food Waste 1	7.70	10,660	7,717	14,500	606
Food Waste 2	7.58	10,783	8,033	13,650	590
Food Waste 3	7.51	10,758	8,083	14,750	592
ZMF 1	8.02	11,198	8,362	11,800	614
ZMF 2	8.27	11,060	8,035	12,400	602
ZMF 3	8.07	11,728	8,488	12,250	607

Table 28: Round 2 BMP post-digestion data

Sample	pH	TS (mg/L)	VS (mg/L)	COD (mg/L)	Ammonia (mg/L)
Seed 1	7.78	6,815	4,250	6700	751
Seed 2	7.74	6,790	4,337	7650	768
Seed 3	7.69	7,063	4,392	6750	785
Carnivore 1	7.56	8,427	5,340	8900	862
Carnivore 2	7.48	8,788	5,295	7750	853
Carnivore 3	7.45	8,680	5,258	7650	885
Primate 1	7.54	8,510	5,708	8950	817
Primate 2	7.61	8,370	5,530	7900	859
Primate 3	7.50	8,920	5,885	8700	836
Hoofstock Mix 1	7.48	8,567	5,450	8200	808
Hoofstock Mix 2	7.52	8,788	5,903	8500	796
Hoofstock Mix 3	7.61	8,790	5,737	7750	798
Bird Mix 1	7.73	7,857	5,060	6850	813
Bird Mix 2	7.76	7,740	5,045	7100	803
Bird Mix 3	7.73	7,585	4,945	7350	747
Zoo Mix 1	7.61	8,285	5,405	7100	780
Zoo Mix 2	7.61	7,650	4,828	7450	789
Zoo Mix 3	7.56	8,063	5,278	8350	766
Food Waste 1	7.41	7,320	4,483	7400	986
Food Waste 2	7.55	7,265	4,512	7900	914
Food Waste 3	7.56	6,965	4,168	7000	860
ZMF 1	7.43	7,935	5,178	8150	802
ZMF 2	7.36	8110	5375	7800	799
ZMF 3	7.37	8217.5	5222.5	8250	796

Table 29: BMP Round 2 gas composition from weekly gas chromatography analysis

Sample	Average Methane (%)	Max Methane (%)	Average CO_2 (%)	Max CO_2 (%)	Average H_2S (%)	Max H_2S (%)
Carnivore	58	71	17	25	382	719
Primate	59	71	21	29	471	682
Hoofstock Mix	57	70	16	26	156	223
Bird Mix	54	70	12	18	111	174
Zoo Mix	58	71	15	24	159	263
Food Waste	57	71	20	26	15	45
ZMF	57	70	18	26	160	243

A3. Round 3 BMP

Table 30: BMP round 3 raw characterization

Sample	TS (mg/kg)	VS (mg/kg)	TS (%)	VS (%)	TS:VS
Filtrate	27,700	20,007	2.8	2.0	0.72
Carnivore	385,655	269,239	38.6	26.9	0.7
Primate	227,454	193,773	22.7	19.4	0.85
Hoofstock Mix	382,058	284,991	38.2	28.5	0.75
Bird Mix	581,304	515,897	58.1	51.6	0.89
Zoo Mix	375,840	283,744	37.6	28.4	0.75
Food Waste	378,684	306,183	37.9	30.6	0.81
ZMF	349,899	259,917	35.0	26.0	0.74

Table 31: Round 3 BMP pre-digestion data

Sample	pH	TS (mg/L)	VS (mg/L)	COD (mg/L)	Ammonia (mg/L)
Seed 1	7.98	8,705	6,155	10,450	191
Seed 2	8.05	8,427	6,145	12,200	105
Seed 3	8.02	8,170	6,147	12,150	128
Carnivore 1	7.65	11,040	8,230	16,650	59
Carnivore 2	8.00	10,605	7,678	11,100	219
Carnivore 3	7.95	10,400	8,055	13,200	30
Primate 1					
Primate 2					
Primate 3					
Hoofstock Mix 1	8.00	9,202	6,290	15,050	233
Hoofstock Mix 2	7.96	8,757	6,082	11,950	179
Hoofstock Mix 3	7.62	8,302	6,097	13,750	181
Bird Mix 1	7.79	9,877	7,282	11,400	150
Bird Mix 2	8.05	11,550	8,777	13,700	12
Bird Mix 3	7.98	9,023	6,562	10,650	75
Zoo Mix 1	7.67	9,002	6,622	11,200	149
Zoo Mix 2	8.10	8,415	6,230	11,050	101
Zoo Mix 3	7.96	9,115	6,983	13,700	87
Food Waste 1	7.93	9,890	7,418	12,850	206
Food Waste 2	7.84	10,005	7,775	14,800	137
Food Waste 3	7.73	10,358	7,950	15,450	127
ZMF 1					
ZMF 2	8.04	10,250	7,282	10,000	63
ZMF 3	8.11	9,797	7,343	12,000	67

Table 32: Round 3 BMP post-digestion data

Sample	pH	TS (mg/L)	VS (mg/L)	COD (mg/L)	Ammonia (mg/L)
Seed 1	6.95	5,215	3,680	6,900	639
Seed 2	7.06	6,113	3,765	8,000	465
Seed 3	7.17	6,245	3,700	6,950	1,003
Carnivore 1	7.13	7,620	4,868	7,000	680
Carnivore 2	7.13	7,388	4,530	7,300	1,115
Carnivore 3	7.02	7,915	4,980	6,700	759
Primate 1					
Primate 2					
Primate 3					
Hoofstock Mix 1	7.24	7,422	4,505	6,600	660
Hoofstock Mix 2	7.08	6,580	4,065	5,700	598
Hoofstock Mix 3	7.08	5,704	3,912	7,200	1,305
Bird Mix 1	7.24	6,213	4,103	6,650	525
Bird Mix 2	7.22	6,464	4,290	6,800	623
Bird Mix 3	7.16	6,770	4,678	5,850	347
Zoo Mix 1	7.07	6,213	4,040	6,450	449
Zoo Mix 2	7.08	6,717	4,525	6,400	369
Zoo Mix 3	7.12	6,783	4,488	6,650	1,460
Food Waste 1	7.29	6,100	3,895	6,900	388
Food Waste 2	7.11	6,785	4,427	6,800	332
Food Waste 3	7.17	6,063	3,925	5,700	580
ZMF 1					
ZMF 2	7.13	7,007	4,440	6,500	819
ZMF 3	7.16	6,845	4,112	10,800	1,270

Table 33: BMP Round 3 gas composition from weekly gas chromatography analysis

Sample	Average Methane (%)	Max Methane (%)	Average CO_2[a] (%)	Max CO_2[a] (%)	Average H_2S (%)	Max H_2S (%)
Carnivore	62	75	19	21	454	1027
Primate						
Hoofstock Mix	61	75	17	21	62	182
Bird Mix	61	75	16	17	227	376
Zoo Mix	58	75	15	19	64	164
Food Waste	64	76	18	19	515	860
ZMF	60	75	16	18	112	196

a. The first week's data point for the CO_2 reading was not measured and is not included in the max or average results.

Appendix C: Additional data from small-scale pilot testing

This appendix provides additional data from the small-scale pilot testing.

C1. Zoo Mix 1

Table 34: Biogas production data for each collection point for zoo mix 1 pilot

Lapsed time (hr)	Lapsed Time (day)	Cumulative Biogas (L)	Biogas Production (L biogas/kg initial VS)	Biogas Production Rate (L biogas/kg initial VS*day)	Pressure (Pascal)
0	0.0	0.0	0.0	0.0	0
18	0.8	2.5	6.7	8.9	1219
22	0.9	2.8	7.5	8.1	1244
24	1.0	2.9	7.7	7.7	1244
73	3.0	4.7	12.5	4.1	1294
88	3.7	4.8	12.8	3.5	100
91	3.8	4.8	12.8	3.4	0
94	3.9	4.8	12.8	3.3	0
96	4.0	4.8	12.8	3.2	0
113	4.7	4.8	12.8	2.7	0
114	4.8	4.8	12.8	2.7	0
119	4.9	4.8	12.8	2.6	0
120	5.0	4.8	12.8	2.6	1244
136	5.7	5.6	14.9	2.6	796
138	5.8	5.7	15.2	2.6	1244
142	5.9	5.9	15.7	2.7	1244
169	7.0	7.2	19.2	2.7	1095
166	6.9	7.5	20.0	2.9	1269
168	7.0	7.6	20.2	2.9	1269
186	7.8	9.0	24.0	3.1	1269
190	7.9	9.4	25.0	3.2	1269
212	8.8	11.3	30.1	3.4	1269
256	10.7	15.9	42.3	4.0	1269
258	10.8	16.1	42.8	4.0	1269
262	10.9	16.5	43.9	4.0	1244
264	11.0	16.6	44.2	4.0	1244
280	11.7	18.5	49.2	4.2	1244
286	11.9	19.1	50.8	4.3	1244
289	12.0	19.4	51.6	4.3	1294
304	12.7	21.1	56.2	4.4	1244
306	12.8	21.4	57.0	4.5	1244
311	12.9	21.8	58.0	4.5	1294
312	13.0	22.0	58.5	4.5	1244
331	13.8	24.2	64.4	4.7	1418
334	13.9	24.5	65.2	4.7	1394

Table 34 (cont’d)

336	14.0	24.7	65.7	4.7	1344
353	14.7	26.7	71.1	4.8	1443
355	14.8	26.9	71.6	4.8	1443
358	14.9	27.4	72.9	4.9	1418
360	15.0	27.6	73.5	4.9	1394
405	16.9	33.2	88.4	5.2	1244
425	17.7	35.8	95.3	5.4	1244
426	17.8	36.1	96.1	5.4	1344
430	17.9	36.6	97.4	5.4	1319
432	18.0	36.9	98.2	5.5	1244
449	18.7	39.1	104.1	5.6	1244
450	18.8	39.2	104.3	5.6	1344
454	18.9	39.7	105.7	5.6	1244
456	19.0	40.0	106.5	5.6	1344
472	19.7	42.0	111.8	5.7	1294
478	19.9	42.7	113.6	5.7	1145
480	20.0	42.9	114.2	5.7	1244
521	21.7	48.8	129.9	6.0	1344
526	21.9	49.3	131.2	6.0	1244
528	22.0	49.6	132.0	6.0	1269
554	23.1	52.7	140.3	6.1	1244
593	24.7	57.6	153.3	6.2	1244
594	24.8	57.7	153.6	6.2	1244
598	24.9	58.2	154.9	6.2	1269
600	25.0	58.4	155.4	6.2	1244
617	25.7	60.4	160.7	6.3	1194
618	25.8	60.8	161.8	6.3	1394
622	25.9	61.0	162.3	6.3	1269
624	26.0	61.2	162.9	6.3	1394
640	26.7	63.2	168.2	6.3	1244
646	26.9	63.8	169.8	6.3	1244
648	27.0	64.1	170.6	6.3	1244
670	27.9	66.6	177.2	6.3	1244
691	28.8	68.9	183.4	6.4	1244
744	31.0	74.8	199.1	6.4	1319
761	31.7	76.4	203.3	6.4	1244
762	31.8	76.4	203.3	6.4	1244
766	31.9	76.9	204.7	6.4	1244
784	32.7	78.7	209.4	6.4	1244
786	32.8	78.9	210.0	6.4	1244
790	32.9	79.3	211.0	6.4	1244
792	33.0	79.5	211.6	6.4	1244
808	33.7	81.3	216.4	6.4	1344
810	33.8	81.5	216.9	6.4	1344

Table 34 (cont'd)

814	33.9	81.9	218.0	6.4	1219
834	34.8	84.1	223.8	6.4	1145
838	34.9	84.4	224.6	6.4	1244
858	35.8	86.4	229.9	6.4	1244
862	35.9	86.7	230.7	6.4	1194
864	36.0	86.9	231.3	6.4	1244
909	37.9	91.0	242.2	6.4	1219
931	38.8	93.0	247.5	6.4	1244
935	38.9	93.3	248.3	6.4	1244
936	39.0	93.4	248.6	6.4	1244
952	39.7	94.9	252.6	6.4	1194
954	39.8	95.0	252.8	6.4	1194
958	39.9	95.4	253.9	6.4	1219
960	40.0	95.6	254.4	6.4	1194
977	40.7	97.0	258.2	6.3	1194
982	40.9	97.4	259.2	6.3	1294
984	41.0	97.6	259.7	6.3	1244
1,004	41.8	99.0	263.5	6.3	1194
1,026	42.8	100.7	268.0	6.3	1095
1,032	43.0	101.2	269.3	6.3	1194
1,077	44.9	104.4	277.8	6.2	1145
1,097	45.7	105.8	281.6	6.2	1145
1,099	45.8	105.9	281.8	6.2	1194
1,102	45.9	106.1	282.4	6.1	1194
1,104	46.0	106.3	282.9	6.2	1194
1,122	46.8	107.4	285.8	6.1	1194
1,126	46.9	107.6	286.4	6.1	1120
1,128	47.0	107.7	286.6	6.1	1145
1,144	47.7	108.6	289.0	6.1	1244
1,146	47.8	108.8	289.6	6.1	1194
1,266	52.8	115.5	307.4	5.8	1145
1,290	53.8	116.6	310.3	5.8	0
1,294	53.9	116.7	310.6	5.8	1070
1,312	54.7	117.5	312.7	5.7	1194
1,314	54.8	117.6	313.0	5.7	796
1,318	54.9	117.7	313.2	5.7	1145
1,339	55.8	118.7	315.9	5.7	1145
1,362	56.8	119.6	318.3	5.6	1145
1,366	56.9	119.6	318.3	5.6	1145
1,368	57.0	119.7	318.6	5.6	1145
1,386	57.8	120.4	320.4	5.5	1194

Table 35: Gas composition measured weekly for zoo mix 1 pilot

Day	Methane (%)	Carbon Dioxide (%)
5	3	6
12	52	35
19	55	35
27	46	31
47	50	37
55	46	32
59	46	33

Table 36: Leachate volume and pH measured as needed from the zoo mix 1 pilot

Day	pH	Volume (mL)
20	8.08	25
23	8.34	22
30	8.37	32
35	8.39	33
48	8.36	46
55	8.67	17
58	8.50	42
59	8.27	16

C2. Zoo Mix 2

Table 37: Biogas production data for each collection point for zoo mix 2 pilot

Lapsed time (hr)	Lapsed Time (day)	Cumulative Biogas (L)	Biogas Production (L biogas/kg initial VS)	Biogas Production Rate (L biogas/kg initial VS*day)	Pressure (Pascal)
0	0.0	0.0	0.0	0.0	0
18	0.8	2.4	6.4	8.6	1170
22	0.9	2.6	7.0	7.7	1244
24	1.0	2.9	7.6	7.6	1194
73	3.0	4.5	12.0	3.9	1344
88	3.7	4.6	12.3	3.3	100
91	3.8	4.6	12.3	3.3	100
94	3.9	4.6	12.3	3.1	100
96	4.0	4.6	12.3	3.1	50
113	4.7	4.6	12.3	2.6	75
114	4.8	4.6	12.3	2.6	50
119	4.9	4.6	12.3	2.5	0
120	5.0	4.6	12.3	2.5	1145
136	5.7	5.4	14.3	2.5	50

Table 37 (cont'd)

138	5.8	5.5	14.6	2.5	1219
142	5.9	5.6	14.9	2.5	1244
169	7.0	6.8	18.2	2.6	1095
166	6.9	7.2	19.0	2.7	1194
168	7.0	7.2	19.0	2.7	1991
186	7.8	8.5	22.5	2.9	1219
190	7.9	8.8	23.4	3.0	1194
212	8.8	10.6	28.1	3.2	1194
256	10.7	15.1	40.1	3.8	1244
258	10.8	15.2	40.4	3.8	1219
262	10.9	15.5	41.3	3.8	1194
264	11.0	15.6	41.6	3.8	1194
280	11.7	17.6	46.8	4.0	1194
286	11.9	18.2	48.3	4.1	1219
289	12.0	18.5	49.2	4.1	1145
304	12.7	20.2	53.9	4.3	1194
306	12.8	20.6	54.7	4.3	1244
311	12.9	21.0	55.9	4.3	1194
312	13.0	21.1	56.2	4.3	1194
331	13.8	23.4	62.4	4.5	1170
334	13.9	23.8	63.2	4.5	1244
336	14.0	24.0	63.8	4.6	1145
353	14.7	26.2	69.7	4.7	1194
355	14.8	26.4	70.3	4.8	1194
358	14.9	27.0	71.7	4.8	1194
360	15.0	27.2	72.3	4.8	1244
405	16.9	33.3	88.7	5.3	1045
425	17.7	36.1	96.0	5.4	1170
426	17.8	36.4	96.9	5.5	1145
430	17.9	37.1	98.7	5.5	1045
432	18.0	37.4	99.5	5.5	1145
449	18.7	39.9	106.3	5.7	1194
450	18.8	40.0	106.6	5.7	1244
454	18.9	40.6	108.0	5.7	1194
456	19.0	40.9	108.9	5.7	1219
472	19.7	43.2	115.1	5.9	1244
478	19.9	44.1	117.4	5.9	1095
480	20.0	44.3	118.0	5.9	1194
521	21.7	50.5	134.4	6.2	1194
526	21.9	51.2	136.1	6.2	1145
528	22.0	51.4	136.7	6.2	1194
554	23.1	55.1	146.7	6.4	1145
593	24.7	60.9	162.2	6.6	1145
594	24.8	61.1	162.5	6.6	1194

Table 37 (cont'd)

598	24.9	61.6	163.9	6.6	1170
600	25.0	61.9	164.8	6.6	1219
617	25.7	64.2	171.0	6.7	1145
618	25.8	64.5	171.6	6.7	1194
622	25.9	65.0	173.0	6.7	1170
624	26.0	65.2	173.6	6.7	1145
640	26.7	67.7	180.0	6.8	1145
646	26.9	68.4	182.1	6.8	1244
648	27.0	68.8	183.0	6.8	1170
670	27.9	71.7	190.9	6.8	1194
691	28.8	74.5	198.2	6.9	1170
744	31.0	81.7	217.5	7.0	1219
761	31.7	83.7	222.8	7.0	1145
762	31.8	83.8	223.1	7.0	1194
766	31.9	84.4	224.5	7.0	1194
784	32.7	86.7	230.7	7.1	1145
786	32.8	86.9	231.3	7.1	1095
790	32.9	87.5	232.7	7.1	1145
792	33.0	87.7	233.3	7.1	1194
808	33.7	90.0	239.5	7.1	1145
810	33.8	90.3	240.3	7.1	1194
814	33.9	90.9	241.8	7.1	1145
834	34.8	93.1	247.7	7.1	1045
838	34.9	93.4	248.5	7.1	1145
858	35.8	95.0	252.9	7.1	1070
862	35.9	95.3	253.5	7.1	1244
864	36.0	95.4	253.8	7.1	1194
909	37.9	98.8	262.9	6.9	1170
931	38.8	100.2	266.7	6.9	1170
935	38.9	100.4	267.3	6.9	1194
936	39.0	100.5	267.6	6.9	1194
952	39.7	101.6	270.5	6.8	1145
954	39.8	101.6	270.5	6.8	1219
958	39.9	102.0	271.4	6.8	1170
960	40.0	102.1	271.7	6.8	1170
977	40.7	103.1	274.3	6.7	1170
982	40.9	103.4	275.2	6.7	1145
984	41.0	103.5	275.5	6.7	995
1,004	41.8	104.4	277.8	6.6	1194
1,026	42.8	105.6	281.0	6.6	946
1,032	43.0	105.9	281.9	6.6	1095
1,077	44.9	107.8	286.9	6.4	995
1,097	45.7	108.6	288.9	6.3	995
1,099	45.8	108.6	288.9	6.3	1045

Table 37 (cont'd)

1,102	45.9	108.7	289.2	6.3	946
1,104	46.0	108.8	289.5	6.3	1045
1,122	46.8	109.3	291.0	6.2	896
1,126	46.9	109.5	291.3	6.2	1269
1,128	47.0	109.6	291.6	6.2	1219
1,144	47.7	110.7	294.5	6.2	1194
1,146	47.8	110.9	295.1	6.2	1145
1,266	52.8	114.5	304.8	5.8	1070
1,290	53.8	115.1	306.2	5.7	0
1,294	53.9	115.2	306.5	5.7	995
1,312	54.7	116.2	309.1	5.7	1170
1,314	54.8	116.2	309.1	5.6	697
1,318	54.9	116.4	309.7	5.6	1045
1,339	55.8	117.8	313.5	5.6	1045
1,362	56.8	118.9	316.5	5.6	1095
1,366	56.9	119.0	316.8	5.6	1941
1,368	57.0	119.1	317.0	5.6	1244
1,386	57.8	119.7	318.5	5.5	1294

Table 38: Gas composition measured weekly for zoo mix 2 pilot

Day	Methane (%)	Carbon Dioxide (%)
5	10	27
12	47	37
19	54	33
27	50	34
47	50	38
55	35	24
59	49	35

Table 39: Leachate volume and pH measured as needed from the Zoo Mix 2 pilot

Day	pH	Volume (mL)
30	8.32	20
35	8.48	33
48	8.27	47
55	8.75	15
58	8.61	26
59	8.25	24

C3. ZMF 1

Table 40: Biogas production data for each collection point for ZMF 1 pilot

Lapsed time (hr)	Lapsed Time (day)	Cumulative Biogas (L)	Biogas Production (L biogas/kg initial VS)	Biogas Production Rate (L biogas/kg initial VS*day)	Pressure (Pascal)
0	0.0	0.0	0.0	0.0	0
16	0.7	1.0	3.0	4.5	1344
32	1.3	1.0	3.0	2.2	348
34	1.4	1.1	3.3	2.3	1344
38	1.6	1.4	4.3	2.7	1319
58	2.4	2.5	7.6	3.2	1145
62	2.6	2.9	8.6	3.3	1319
64	2.7	2.9	8.6	3.2	1319
82	3.4	3.6	11.0	3.2	1294
86	3.6	3.9	11.6	3.2	1319
108	4.5	4.6	13.9	3.1	1194
152	6.3	6.2	18.6	2.9	1269
154	6.4	6.2	18.6	2.9	1219
158	6.6	6.2	18.6	2.8	1344
160	6.7	6.3	18.9	2.8	1344
176	7.3	6.8	20.6	2.8	1294
182	7.6	6.9	20.9	2.8	1369
185	7.7	7.0	21.2	2.8	1394
200	8.3	7.6	22.9	2.7	1344
202	8.4	7.6	22.9	2.7	1294
207	8.6	7.7	23.2	2.7	1244
208	8.7	7.7	23.2	2.7	1344
227	9.5	8.4	25.2	2.7	1170
230	9.6	8.5	25.6	2.7	1344
232	9.7	8.5	25.6	2.6	1145
249	10.4	9.1	27.6	2.7	1194
251	10.4	9.2	27.9	2.7	1344
254	10.6	9.4	28.2	2.7	1344
256	10.7	9.5	28.6	2.7	1294
301	12.5	11.3	34.2	2.7	1095
321	13.4	12.3	37.2	2.8	1344
322	13.4	12.4	37.5	2.8	1344
326	13.6	12.7	38.2	2.8	1194
328	13.7	12.8	38.5	2.8	1294
345	14.4	13.8	41.5	2.9	1344
346	14.4	13.8	41.5	2.9	1344
350	14.6	13.9	41.8	2.9	1344
352	14.7	14.0	42.2	2.9	1294

Table 40 (cont'd)

368	15.3	14.9	44.8	2.9	1344
374	15.6	15.2	45.8	2.9	1194
376	15.7	15.3	46.2	2.9	1344
417	17.4	17.8	53.8	3.1	1344
422	17.6	18.0	54.5	3.1	1344
424	17.7	18.3	55.1	3.1	1344
450	18.7	19.8	59.8	3.2	1344
489	20.4	22.9	69.1	3.4	1294
490	20.4	23.0	69.4	3.4	1294
494	20.6	23.2	70.1	3.4	1369
496	20.7	23.4	70.7	3.4	1344
513	21.4	24.8	74.7	3.5	1344
514	21.4	24.8	74.7	3.5	1294
518	21.6	25.1	75.7	3.5	1319
520	21.6	25.2	76.0	3.5	1319
536	22.3	26.5	80.0	3.6	1294
542	22.6	27.0	81.3	3.6	1319
544	22.7	27.2	82.0	3.6	1319
566	23.6	28.9	87.3	3.7	1294
587	24.4	30.7	92.6	3.8	1319
640	26.7	35.6	107.6	4.0	1294
657	27.4	37.2	112.2	4.1	1294
658	27.4	37.2	112.2	4.1	1294
662	27.6	37.6	113.6	4.1	1244
680	28.3	39.4	118.9	4.2	1244
682	28.4	39.6	119.5	4.2	1194
686	28.6	40.0	120.9	4.2	1244
688	28.7	40.2	121.2	4.2	1219
704	29.3	41.9	126.5	4.3	1244
706	29.4	42.1	127.2	4.3	1244
710	29.6	42.5	128.2	4.3	1244
730	30.4	44.7	134.8	4.4	1194
734	30.6	45.0	135.8	4.4	1244
754	31.4	47.1	142.1	4.5	1244
758	31.6	47.4	143.1	4.5	1219
760	31.7	47.5	143.4	4.5	1244
805	33.5	51.8	156.4	4.7	1244
827	34.4	53.8	162.4	4.7	1244
831	34.6	54.1	163.4	4.7	1244
832	34.7	54.3	164.0	4.7	1244
848	35.3	55.9	168.7	4.8	1244
850	35.4	56.0	169.0	4.8	1244
854	35.6	56.4	170.3	4.8	1244
856	35.7	56.5	170.7	4.8	1344

Table 40 (cont'd)

873	36.4	58.1	175.3	4.8	1369
878	36.6	58.5	176.6	4.8	1344
880	36.7	58.6	177.0	4.8	1319
900	37.5	60.2	181.6	4.8	1394
902	37.6	60.4	182.3	4.9	1344
922	38.4	62.0	187.3	4.9	1194
928	38.7	62.2	187.6	4.9	1294
973	40.5	65.3	197.2	4.9	1194
993	41.4	66.7	201.2	4.9	1294
995	41.4	66.9	201.9	4.9	1344
998	41.6	67.1	202.5	4.9	1344
1001	41.7	67.3	203.2	4.9	1344
1018	42.4	68.4	206.5	4.9	1344
1022	42.6	68.8	207.5	4.9	1369
1024	42.7	68.9	207.8	4.9	1319
1040	43.3	69.9	210.8	4.9	1344
1042	43.4	70.0	211.2	4.9	1344
1162	48.4	76.8	231.8	4.8	1294
1186	49.4	78.0	235.4	4.8	1443
1190	49.6	78.2	236.1	4.8	0
1208	50.3	78.5	237.1	4.7	149
1210	50.4	78.5	237.1	4.7	199
1214	50.6	78.7	237.4	4.7	1170
1235	51.5	79.4	239.7	4.7	149
1258	52.4	79.4	239.7	4.6	224
1262	52.6	79.5	240.1	4.6	1120
1264	52.7	79.5	240.1	4.6	1194
1282	53.4	80.6	243.4	4.6	1145

Table 41: Gas composition measured weekly for ZMF 1 pilot

Day	Methane (%)	Carbon Dioxide (%)
7	21	49
14	47	41
22	55	34
32	56	33
39	54	-
42	56	34
50	51	30
54	55	24

Table 42: Leachate volume and pH measured as needed from the ZMF 1 pilot

Day	pH	Volume (mL)
15	6.04	32
18	6.52	32
22	7.29	35
25	7.78	29
30	8.18	30
35	8.50	38
42	8.40	40
50	8.59	43
53	8.04	-
54	8.40	13

C4. ZMF 2

Table 43: Biogas production data for each collection point for ZMF 2 pilot

Lapsed time (hr)	Lapsed Time (day)	Cumulative Biogas (L)	Biogas Production (L biogas/kg initial VS)	Biogas Production Rate (L biogas/kg initial VS*day)	Pressure (Pascal)
0	0.0	0.0	0.0	0.0	0
16	0.7	1.2	3.7	5.5	1344
32	1.3	3.1	9.3	7.0	1344
34	1.4	3.2	9.6	6.8	1294
38	1.6	3.5	10.6	6.7	1294
58	2.4	4.5	13.6	5.6	1145
62	2.6	4.7	14.3	5.5	1319
64	2.7	4.7	14.3	5.4	1617
82	3.4	5.5	16.6	4.9	1369
86	3.6	5.7	17.3	4.8	1344
108	4.5	6.4	19.3	4.3	1294
152	6.3	7.6	22.9	3.6	1294
154	6.4	7.7	23.2	3.6	1219
158	6.6	7.7	23.2	3.5	1244
160	6.7	7.7	23.2	3.5	1269
176	7.3	8.1	24.6	3.4	1244
182	7.6	8.3	24.9	3.3	1194
185	7.7	8.3	24.9	3.2	1294
200	8.3	8.6	25.9	3.1	1294
202	8.4	8.7	26.2	3.1	1194
207	8.6	8.8	26.6	3.1	1194
208	8.7	8.8	26.6	3.1	1244
227	9.5	9.1	27.6	2.9	1045

Table 43 (cont'd)

230	9.6	9.2	27.9	2.9	1219
232	9.7	9.2	27.9	2.9	1045
249	10.4	9.6	28.9	2.8	1095
251	10.4	9.7	29.2	2.8	1294
254	10.6	9.8	29.6	2.8	1294
256	10.7	9.8	29.6	2.8	1194
301	12.5	10.9	32.9	2.6	1045
321	13.4	11.6	34.9	2.6	1145
322	13.4	11.6	34.9	2.6	1294
326	13.6	11.7	35.2	2.6	1095
328	13.7	11.8	35.5	2.6	1194
345	14.4	12.3	37.2	2.6	1244
346	14.4	12.3	37.2	2.6	1244
350	14.6	12.4	37.5	2.6	1194
352	14.7	12.5	37.9	2.6	1145
368	15.3	13.0	39.2	2.6	1244
374	15.6	13.1	39.5	2.5	1070
376	15.7	13.2	39.8	2.5	1244
417	17.4	14.4	43.5	2.5	1244
422	17.6	14.5	43.8	2.5	1294
424	17.7	14.6	44.2	2.5	1294
450	18.7	15.4	46.5	2.5	1244
489	20.4	16.9	51.1	2.5	1244
490	20.4	16.9	51.1	2.5	1244
494	20.6	17.1	51.5	2.5	1244
496	20.7	17.2	51.8	2.5	1244
513	21.4	17.7	53.5	2.5	1244
514	21.4	17.7	53.5	2.5	796
518	21.6	17.8	53.8	2.5	1269
520	21.6	17.9	54.1	2.5	1294
536	22.3	18.5	55.8	2.5	1244
542	22.6	18.7	56.4	2.5	1244
544	22.7	18.8	56.8	2.5	1294
566	23.6	19.6	59.1	2.5	1244
587	24.4	20.4	61.4	2.5	1269
640	26.7	22.7	68.4	2.6	1244
657	27.4	23.3	70.4	2.6	1244
658	27.4	23.3	70.4	2.6	1294
662	27.6	23.5	71.1	2.6	1244
680	28.3	24.3	73.4	2.6	1244
682	28.4	24.4	73.7	2.6	1244
686	28.6	24.6	74.4	2.6	1244
688	28.7	24.8	74.7	2.6	1244
704	29.3	25.6	77.4	2.6	1244

Table 43 (cont'd)

706	29.4	25.7	77.7	2.6	1244
710	29.6	26.0	78.4	2.6	1244
730	30.4	27.1	81.7	2.7	1244
734	30.6	27.3	82.3	2.7	1194
754	31.4	28.4	85.7	2.7	1269
758	31.6	28.5	86.0	2.7	1244
760	31.7	28.6	86.3	2.7	1244
805	33.5	30.7	92.6	2.8	1244
827	34.4	31.8	96.0	2.8	1244
831	34.6	32.0	96.6	2.8	1269
832	34.7	32.2	97.3	2.8	1269
848	35.3	33.0	99.6	2.8	1294
850	35.4	33.1	99.9	2.8	1244
854	35.6	33.3	100.6	2.8	1244
856	35.7	33.4	100.9	2.8	1244
873	36.4	34.3	103.6	2.8	1369
878	36.6	34.7	104.6	2.9	1219
880	36.7	34.8	104.9	2.9	1244
900	37.5	35.6	107.6	2.9	1244
902	37.6	35.8	107.9	2.9	1244
922	38.4	36.9	111.2	2.9	1145
928	38.7	37.2	112.2	2.9	1294
973	40.5	39.5	119.2	2.9	1194
993	41.4	40.5	122.2	3.0	1244
995	41.4	40.6	122.5	3.0	1244
998	41.6	40.8	123.2	3.0	1244
1001	41.7	41.0	123.8	3.0	1244
1018	42.4	41.8	126.2	3.0	1244
1022	42.6	41.9	126.5	3.0	1394
1024	42.7	42.0	126.8	3.0	1344
1040	43.3	42.5	128.2	3.0	1344
1042	43.4	42.8	129.2	3.0	1319
1162	48.4	48.0	144.8	3.0	1319
1186	49.4	49.0	147.8	3.0	597
1190	49.6	49.1	148.1	3.0	771
1208	50.3	49.7	150.1	3.0	1344
1210	50.4	49.8	150.4	3.0	1095
1214	50.6	49.9	150.7	3.0	1344
1235	51.5	50.2	151.4	2.9	1244
1258	52.4	50.4	152.1	2.9	1344
1262	52.6	50.4	152.1	2.9	1145
1264	52.7	50.4	152.1	2.9	747
1282	53.4	50.4	152.1	2.8	846

Table 44: Gas composition measured weekly for ZMF 2 pilot

Day	Methane (%)	Carbon Dioxide (%)
7	19	52
14	40	46
22	53	39
32	57	37
39	52	
42	57	34
50	29	18
54	60	25

Table 45: Leachate volume and pH measured as needed from the ZMF 2 pilot

Day	pH	Volume (mL)
15	6.38	34
18	6.58	40
22	6.60	28
25	7.05	38
30	7.52	36
42	8.18	42
43	8.08	42
50	8.53	30
54	8.44	16

Appendix D: MATLAB code for fitting model to the small-scale pilot digester data

This appendix provides the code used for fitting the the Modified Gompertz equation model to the data from the small-scale pilot digesters.

```
%% Housekeeping
close all
clear
clc
format long

%% Read in data
data=xlsread('zoo model data');

%% Experimental Data
tobs=data(:,1);
nobs=length(tobs);
yobs=data(:,2);
figure
plot(tobs, yobs,'o')
xlabel 'Time (d)'
ylabel 'Cumulative Biogas Production (L/kg initial VS)'

%% Explicit Function
fnameFOR=@Gompertz_FOR;
type Gompertz_FOR.m

%% Initial Guesses
P= 300;
Rm= 9;
lambda= 8;

beta0(1)=P;
beta0(2)=Rm;
beta0(3)=lambda;
p=length(beta0);

%% Call and plot the function
Y=Gompertz_FOR(beta0,tobs);
plot(tobs,Y)

%% nlinfit returns parameters, residuals, Jacobian (sensitivity coefficient matrix),
fnameINV=@Gompertz_FOR;
[beta,resids,J,COVB,mse] = nlinfit(tobs, yobs,fnameINV, beta0);
beta
rmse=sqrt(mse)
```

```
condX=cond(J)
detXTX=det(J'*J)
[R,sigma]=corrcov(COVB);
R
sigma
relerr=sigma'./beta
ci=nlparci(beta,resids,J)
meanr=mean(resids)

%% Plot of Observed Data and Predicted Model
ypred=fnameINV(beta,tobs);
figure
plot(tobs,ypred)
hold on
plot(tobs,yobs,'o')
ylabel 'Biogas (L per kg initial VS)'
xlabel 'Time (d)'
legend ('Predicted', 'Observed', 'location', 'best')

%% X' = scaled sensitivity coefficients using forward-difference for estimated parameters
ts=linspace(0,max(tobs),1000)';
ypred=fnameINV(beta,ts);
Xp=SSC_V3(beta,ts,fnameFOR);
ns=length(ts);
cmap = ['r' 'g' 'b' 'c' 'y'  'm' 'k' ]';
figure
hold on
set(gca, 'fontsize',14,'fontweight','bold');
h2(1)=plot(ts,ypred,'-','color',cmap(1,:),'LineWidth',2);
for i=1:p
   h2(i+1) = plot(ts,Xp(:,i),'-','color',cmap(i+1,:),'LineWidth',2);
end
legend('Predicted','P','R_m','\lambda', 'location', 'best')
xlabel('Time (d)'); ylabel('Scaled Sensitivity Coefficient or L biogas/kg initial VS');
grid on

%% Confidence and prediction intervals for the dependent variable
[ypred, delta] = nlpredci(fnameINV,tobs,beta,resids,J,0.05,'on','curve');
[ypred, deltaob] =nlpredci(fnameINV,tobs,beta,resids,J,0.05,'on','observation');
CBu=ypred+delta;
CBl=ypred-delta;
PBu=ypred+deltaob;
PBl=ypred-deltaob;

figure
hold on
```

```
plot(tobs,ypred,'-b');
plot(tobs,yobs,'sb','MarkerFaceColor','b');
plot(tobs,CBu,'--r','LineWidth',1);
plot(tobs,CBl,'--r','LineWidth',1);
plot(tobs,PBu,'-.m','LineWidth',1);
plot(tobs,PBl,'-.m','LineWidth',1);
legend('ypred','yobs','CB','','PB','','location','best')
xlabel 'Time (d)'
ylabel 'Cumulative Biogas Production (L biogas/kg initial VS)'

%% residual scatter plot
figure
hold on
n=length(tobs);
plot(tobs(:,1), resids(1:n), 'sb','Markerfacecolor', 'b')
YLine = [0 0];
XLine = [0 60];
plot (XLine, YLine,'R');
ylabel('Observed M - Predicted M','fontsize',14,'fontweight','bold')
xlabel('Time (d)','fontsize',14,'fontweight','bold')
legend('Residuals','location','best')

%% residual histogram
figure
normhist(resids);
[n1, xout] = hist(resids,10);
figure
hold on
set(gca, 'fontsize',14,'fontweight','bold');
bar(xout, n1)
xlabel('Observed y/\sigma - Predicted y/\sigma','fontsize',16,'fontweight','bold')
ylabel('Frequency','fontsize',16,'fontweight','bold')

%% prior information
b_old=beta0';
p=length(b_old);
sig=25;
sig=sig*ones(n,1);
tol=5e-4;
ratio = 1;
d=0.001;
count=1;

%% start sequential estimation
while ratio>tol
b= b_old;
```

```
ypred=Gompertz_FOR(b,tobs);
e=yobs-ypred;

for i=1:length(b)
   bin=b;
   bin(i)=b(i)*(1+d);
   yhat{i}=Gompertz_FOR(bin,tobs);
   XX{i}=(yhat{i}-ypred)/(b(i)*d);
   if i==1
      X=XX{i};
   else
      X=[X XX{:,i}];
   end
end


P=10*[b_old(1)^2 0 0; 0 b_old(2)^2 0; 0 0 b_old(3)^2];
B=b_old';
for ii=1:n;
   A=P*X(ii,:)';
   Delta=sig(ii)^2+X(ii,:)*A;
   K=A/Delta;
   b=b+K*(e(ii)-X(ii,:)*(b-b_old));
   P=P-K*A';
   B=[B;b'];
   if ii==1
      PP=[P(1,1) P(1,2) P(2,2)];
   else
      PP=[PP; P(1,1) P(1,2) P(2,2)];
   end
end
b_new=b;
ratio=max(abs((b_new-b_old)./b_old));

b_old=b_new;
count=count+1;
end
BB=B(2:end,:);

%% Compute final sensitivity matrix
b= b_old;
ypred=Gompertz_FOR(b,tobs);
e=yobs-ypred;
for i=1:length(b)
   bin=b;
   bin(i)=b(i)*(1+d);
```

```
    yhat{i}=Gompertz_FOR(bin,tobs);
    XX{i}=(yhat{i}-ypred)/(b(i)*d);
    if i==1
        X=XX{i};
    else
        X=[X XX{:,i}];
    end
end

%% results
b_new
sigma=sqrt(diag(P))
relerr=sigma./b_new
mse=e'*e/(n-p);
rmse=sqrt(mse)

%% sequential plots
figure
hold on
plot(tobs,BB(:,1),'sg','markerfacecolor','b')
plot(tobs,BB(:,2),'ob','markerfacecolor','r')
plot(tobs,BB(:,3),'oc','markerfacecolor','g')
xlabel('Cumulative Biogas Production (L biogas/kg initial VS)')
ylabel('Parameter')
grid on

%% sequential normalized plots
BBn=BB(:,1)./BB(end,1);
BBn(:,2)=BB(:,2)./BB(end,2);
BBn(:,3)=BB(:,3)./BB(end,3);
figure
plot(tobs,BBn(:,1),'-g', 'linewidth',1.8)
hold on
plot(tobs,BBn(:,2),'-b','linewidth',1.8)
plot(tobs,BBn(:,3),'-c','linewidth',1.8)
xlabel('Time (d)')
ylabel('Normalized Parameter')
legend('P','R_m','\lambda', 'location', 'best')
grid on

%% Bootstrapping
%% nlinfit returns beta, residuals, Jacobian (sensitivity coefficient matrix),
%covariance matrix, and mean square error
t=tobs;
[beta,resids,J,COVB,mse] = nlinfit(t,yobs,@Gompertz_FOR,beta0);
rmse=sqrt(mse);
```

```
%% R is the correlation matrix for the betaeters, sigma is the standard deviation vector
[R,sigma]=corrcov(COVB);

%% asymptotic confidence intervals for beta
ci95=nlparci(beta,resids,J);
ci90=nlparci(beta,resids,J, 0.1);

%% nonlinear regression confidence intervals-- 'on' means simultaneous
[ypred, delta] = nlpredci('Gompertz_FOR',t,beta,resids,J,0.05,'on','curve');
[ypred, deltaob] =nlpredci('Gompertz_FOR',t,beta,resids,J,0.05,'on','observation');

%% simultaneous confidence bands for regression line
CBu=ypred+delta;
CBl=ypred-delta;

%% simultaneous prediction bands for regression line
PBu=ypred+deltaob;
PBl=ypred-deltaob;

%% bootstrap CI for beta
nboot=1000;
betab(1,:)=beta;
ypredb(1,:)=ypred;

mm=2;
for j=2:nboot
   r=round(1 + (n-1).*rand(n,1));
   for i=1:n
      if mm==1
         tt(i)=t(r(i));
         yboot(i)=yobs(r(i));
      end
      if mm==2
         tt=t;
         yboot(i)=ypred(i)+resids(r(i));
         if i==n
            yboot=yboot';
         end
      end
   end
   [betab(j,:),rr(j,:),J2,COVB2,mse2]= nlinfit(tt,yboot,'Gompertz_FOR',beta0);
   ypredb(j,:)=Gompertz_FOR(betab(j,:),t);
   clear yboot
end
r2=rr(1,:)';
```

```
for j=2:nboot
   r2=[r2; rr(j,:)'];
end
bsort=sort(betab,1); ysort=sort(ypredb,1);
L=round(0.025*nboot);
if L==0;  L=1;  end
U=round(0.975*nboot);
cib(1,1)=bsort(L,1); cib(1,2)=bsort(U,1);
cib(2,1)=bsort(L,2); cib(2,2)=bsort(U,2);
for i=1:n
   ybci(i,1)=ysort(L,i); ybci(i,2)=ysort(U,i);
end
%% compute bootstrap prediction bands
D=rmse*tinv(.975,n-p);
CIwb(:,1)=ybci(:,1)-ypred; CIwb(:,2)=ypred-ybci(:,2);
PIwb(:,1)=sqrt(CIwb(:,1).^2+D^2); PIwb(:,2)=sqrt(CIwb(:,2).^2+D^2);
PIb(:,1)=ypred+PIwb(:,1); PIb(:,2)=ypred-PIwb(:,2);

%% residual histogram for bootstrap residuals
[n1, xout] = hist(r2,6);
figure
hold on
set(gca, 'fontsize',14,'fontweight','bold');
bar(xout, n1)
xlabel('Observed M-Predicted M','fontsize',16,'fontweight','bold')
ylabel('Frequency','fontsize',16,'fontweight','bold')

figure
hold on
set(gca, 'fontsize',14,'fontweight','bold');
L4 = ['Time (d)'];
xlabel(L4,'fontsize',16,'fontweight','bold');
ylabel('Cumulative Biogas Production (L/kg initial VS)','fontsize',16,'fontweight','bold');
h1(1)=plot(t,yobs,'square', 'Markerfacecolor', 'b');
h1(2) = plot(t,ypred,'-','LineWidth',1);
h1(3) = plot(t,CBu,'--r','LineWidth',1);
plot(t,CBl,'--r','LineWidth',1);

%% plot prediction band for regression line
h1(4) = plot(t,PBu,'-.m','LineWidth',1);
plot(t,PBl,'-.m','LineWidth',1);

%% plot bootstrap bands
h1(5) = plot(t,ybci(:,1),'--k','LineWidth',1);
plot(t,ybci(:,2),'--k','LineWidth',1);
h1(6) = plot(t,PIb(:,1),'-g','LineWidth',1);
```

```
plot(t,PIb(:,2),'-g','LineWidth',1);
legend(h1,'Biogasobs','Biogaspred','asyCB','asyPB','bootCB','bootPB','location','best')

meanres=mean(resids);
%% residual scatter plot
figure
hold on
set(gca, 'fontsize',14,'fontweight','bold');
plot(t, resids, 'square', 'Markerfacecolor', 'b')
plot([0,max(t)],[0,0], 'R')
ylabel('Observed M-Predicted M','fontsize',16,'fontweight','bold')
xlabel('Time (d)','fontsize',16,'fontweight','bold')

%% residual histogram
[n1, xout] = hist(resids,6);
figure
hold on
set(gca, 'fontsize',14,'fontweight','bold');
bar(xout, n1)
xlabel('Observed M-Predicted M','fontsize',16,'fontweight','bold')
ylabel('Frequency','fontsize',16,'fontweight','bold')
```

REFERENCES

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2012 Summit Aquariums Communicating Climate Change. (2011, September 12-17). Paper presented at the 2011 Annual AZA Conference, Atlanta, Georgia.

Agstar. (2011). *Common Safety Practices for On-Farm Anaerobic Digestion Systems*.

America, the Ocean, and Climate Change: New Research Insights for Conservation, Awareness, and Action. (2009). In T. O. Project (Ed.).

Appels, L., Lauwers, J., Degrève, J., Helsen, L., Lievens, B., Willems, K., . . . Dewil, R. (2011). Anaerobic digestion in global bio-energy production: Potential and research challenges. *Renewable and Sustainable Energy Reviews, 15*(9), 4295-4301. doi:10.1016/j.rser.2011.07.121

Association of Zoos and Aquariums. (2016). Retrieved from www.aza.org

Badshah, M., Lam, D. M., Liu, J., & Mattiasson, B. (2012). Use of an Automatic Methane Potential Test System for evaluating the biomethane potential of sugarcane bagasse after different treatments. *Bioresour Technol, 114*, 262-269. doi:10.1016/j.biortech.2012.02.022

Bracmort, K., Burns, R. T., Beddoes, J., & Lazarus, W. (2008). *An Analysis of Anaerobic Digestion System Costs on U.S. Livestock Production Systems*. Paper presented at the 2008 ASABE Annual International Meeting, Providence, Rhode Island.

Brule, M., Oechsner, H., & Jungbluth, T. (2014). Exponential model describing methane production kinetics. *Bioprocess Biosyst Eng, 37*, 1759-1770. doi:10.1007/s00449-014-1150-4

Chynoweth, D. P., Turick, C. E., Owens, J. M., Jerger, D. E., & Peck, M. W. (1993). Biochemical methane potential of biomass and waste feedstocks. *Biomass and Bioenergy, 5*(1), 95-111.

Conservation Practice Standard Anaerobic Digester Code 366. (2009).

Cost-Effectiveness Biogas Calculator. (2016). *Online European Feedstock Atlas Basis Version*. Retrieved from http://daten.ktbl.de/euagrobiogasbasis/substratemischung.do?selectedSubstrate=Chip+fat+&selectedAction=substrateGruppe

Crook, M. F., & Gould, C. M. (2009). *On Farm Anaerobic Digester Handbook*. Michgan State University Extension, Michigan Department of Agriculture. Michigan State University.

Detroit Zoo. (2016). Retrieved from https://detroitzoo.org/about/your-detroit-zoo/

Deublein, D., & Steinhauser, A. (2008). Biogas *Biogas from Waste and Renewable Resources: An Introduction.*

Donoso-Bravo, A., Bandara, W. M., Satoh, H., & Ruiz-Filippi, G. (2013). Explicit temperature-based model for anaerobic digestion: application in domestic wastewater treatment in a UASB reactor. *Bioresour Technol, 133*, 437-442. doi:10.1016/j.biortech.2013.01.174

Donoso-Bravo, A., Pérez-Elvira, S. I., & Fdz-Polanco, F. (2010). Application of simplified models for anaerobic biodegradability tests. Evaluation of pre-treatment processes. *Chemical Engineering Journal, 160*(2), 607-614. doi:10.1016/j.cej.2010.03.082

Ebner, J. H., Labatut, R. A., Lodge, J. S., Williamson, A. A., & Trabold, T. A. (2016). Anaerobic co-digestion of commercial food waste and dairy manure: Characterizing biochemical parameters and synergistic effects. *Waste Manag, 52*, 286-294. doi:10.1016/j.wasman.2016.03.046

El Achkar, J. H., Lendormi, T., Hobaika, Z., Salameh, D., Louka, N., Maroun, R. G., & Lanoiselle, J. L. (2016). Anaerobic digestion of grape pomace: Biochemical characterization of the fractions and methane production in batch and continuous digesters. *Waste Manag, 50*, 275-282. doi:10.1016/j.wasman.2016.02.028

EPA. (2010a). Greenhouse gas emissions estimation methodologies for biogenic emissions from selected source categories: solid waste disposal, wastewater treatment, and ethanol fermentation. .

EPA. (2010b). IPCC guidelines for national greenhouse gas inventories. .

EPA. (2014). eGRID 9th edition Version 1.0 Subregion File (Year 2010 Data).

EPA. (2016). AgSTAR: Biogas Recovery in the Agriculture Sector. Retrieved from https://www.epa.gov/agstar

EPA. (2017). Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2011, Annex 2, Table A-38. Retrieved from http://www.epa.gov/climatechange/ghgemissions/usinventoryreport.html

Faivor, L. L., & Kirk, D. (2011). *Statistical Verification of a Biochemical Methane Potential Test*. Paper presented at the 2011 ASABE Annual International Meeting, Louisville, Kentucky.

Garcia-Ochoa, F., Santos, V. E., Naval, L., Guardiola, E., & Lopez, B. (1999). Kinetic model for anaerobic digestion of livestock manure. *Enzyme and Microbial Technology, 25*, 55-60.

Glover, T. J. (1995). *Pocket Ref* (Second ed.): Sequoia Publishing, Inc.

Greenprint. (2016). Retrieved from https://detroitzoo.org/about/greenprint-sustainability/

Handbury, R. (2016, 8/25/2016). [Food waste estimates].

Hansen, T. L., Schmidt, J. E., Angelidaki, I., Marca, E., Jansen, J. l. C., Mosbæk, H., . . . Institutionen för, k. (2004). Method for determination of methane potentials of solid organic waste. *Waste Management, 24*(4), 393-400. doi:10.1016/j.wasman.2003.09.009

Hidalgo, D., & Martín-Marroquín, J. M. (2015). Biochemical methane potential of livestock and agri-food waste streams in the Castilla y León Region (Spain). *Food Research International, 73,* 226-233. doi:10.1016/j.foodres.2014.12.044

Kafle, G. K., & Chen, L. (2016). Comparison on batch anaerobic digestion of five different livestock manures and prediction of biochemical methane potential (BMP) using different statistical models. *Waste Manag, 48,* 492-502. doi:10.1016/j.wasman.2015.10.021

Kelsey, E. (2010). *Forming a National Coalition of Aquariums Focusing on Climate Change and Oceans.* Paper presented at the 2010 Annual AZA Conference Houston, Texas.

Kerr, D. (2010). *Become a Social Change Agent and Build Rewards, Recognition, and Relevance.* Paper presented at the 2010 Annual AZA Conference, Houston, Texas.

Khalid, A., Arshad, M., Anjum, M., Mahmood, T., & Dawson, L. (2011). The anaerobic digestion of solid organic waste. *Waste Manag, 31*(8), 1737-1744. doi:10.1016/j.wasman.2011.03.021

Klasson, K. T., & Nghiem, N. P. (2003). *Energy Production from Zoo Animal Waste*. (ORNL/TM-2003/51).

Kondaveeti, S., & Min, B. (2015). Bioelectrochemical reduction of volatile fatty acids in anaerobic digestion effluent for the production of biofuels. *Water Res, 87,* 137-144. doi:10.1016/j.watres.2015.09.011

Kurchania, A., Panwar, N. L., & D. Pagar, S. (2011). *Improved Biogas Stove with Scrubbing Unit for Household Use* (Vol. 2).

Kusch, S. (2012). *Organic Wastes and Energy Source in Zoos*. Paper presented at the 4th International Conference on Engineering for Waste and Biomass Valorisation, Porto, Portugal.

Lay, J.-J., Li, Y.-Y., & Noike, T. (1997). Influences of pH and moisture content on the methane production in high-solids sludge digestion. *Water Research, 31*(6), 1518-1524. doi:10.1016/s0043-1354(96)00413-7

Liu, C. F., Yuan, X. Z., Zeng, G. M., Li, W. W., & Li, J. (2008). Prediction of methane yield at optimum pH for anaerobic digestion of organic fraction of municipal solid waste. *Bioresour Technol, 99*(4), 882-888. doi:10.1016/j.biortech.2007.01.013

Local energy production from biowaste. (2014). Bioferm.

López, I., Passeggi, M., & Borzacconi, L. (2015). Validation of a simple kinetic modelling approach for agro-industrial waste anaerobic digesters. *Chemical Engineering Journal, 262*, 509-516. doi:10.1016/j.cej.2014.10.003

Lorimor, J., Powers, W., & Sutton, A. (2004). Manure Characteristics Manure Management Systems Series.

Maclellan, J., Chen, R., Kraemer, R., Zhong, Y., Liu, Y., & Liao, W. (2013). Anaerobic treatment of lignocellulosic material to co-produce methane and digested fiber for ethanol biorefining. *Bioresour Technol, 130*, 418-423. doi:10.1016/j.biortech.2012.12.032

Mani, S., Sundaram, J., & Das, K. C. (2016). Process simulation and modeling: Anaerobic digestion of complex organic matter. *Biomass and Bioenergy, 93*, 158-167. doi:10.1016/j.biombioe.2016.07.018

Martins, L. F., Antunes, L. P., Pascon, R. C., de Oliveira, J. C., Digiampietri, L. A., Barbosa, D., . . . Setubal, J. C. (2013). Metagenomic analysis of a tropical composting operation at the sao paulo zoo park reveals diversity of biomass degradation functions and organisms. *PLoS One, 8*(4), e61928. doi:10.1371/journal.pone.0061928

McMaster-Carr. (2018). Retrieved from https://www.mcmaster.com/

Mes, T. Z. D. d., Stams, A. J. M., Reith, J. H., & Zeeman, G. (2003). Methane production by anaerobic digestion of wastewater and solid wastes.

Olsen, A. E., & Caruana, D. J. (2011). *Anaerobic Digestion : Processes, Products, and Applications*. Hauppauge, N.Y.: Nova Science Publishers, Inc.

Roos, K. F., Martin Jr., J. B., & Moser, M. A. (2004). *A Manual For Developing Biogas Systems at Commercial Farms in the United States*. AgSTAR Handbook.

Simon, L. Wie Zoos Energiefressern den Riegel vorschieben.

Sliz-Szkliniarz, B., & Vogt, J. (2012). A GIS-based approach for evaluating the potential of biogas production from livestock manure and crops at a regional scale: A case study for the Kujawsko-Pomorskie Voivodeship. *Renewable & Sustainable Energy Reviews, 16*(1), 752-763. doi:10.1016/j.rser.2011.09.001

Sun, Q., Li, H., Yan, J., Liu, L., Yu, Z., & Yu, X. (2015). Selection of appropriate biogas upgrading technology-a review of biogas cleaning, upgrading and utilisation. *Renewable and Sustainable Energy Reviews, 51*, 521-532. doi:10.1016/j.rser.2015.06.029

Tambone, F., Scaglia, B., D'Imporzano, G., Schievano, A., Orzi, V., Salati, S., & Adani, F. (2010). Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. *Chemosphere, 81*(5), 577-583. doi:10.1016/j.chemosphere.2010.08.034

Thamsiriroj, T., Nizami, A. S., & Murphy, J. D. (2012). Use of modeling to aid design of a two-phase grass digestion system. *Bioresour Technol, 110*, 379-389. doi:10.1016/j.biortech.2012.01.113

Understanding Global Warming Potentials. (2016). *Greenhouse Gas Emissions.* Retrieved from https://www.epa.gov/ghgemissions/understanding-global-warming-potentials

Wang, X., Lu, X., Li, F., & Yang, G. (2014). Effects of temperature and carbon-nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: focusing on ammonia inhibition. *PLoS One, 9*(5), e97265. doi:10.1371/journal.pone.0097265

Ward, A. J., Hobbs, P. J., Holliman, P. J., & Jones, D. L. (2008). Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technology, 99*(17), 7928-7940. doi:10.1016/j.biortech.2008.02.044

Water, R. (2016). Anaerobic Wastewater Treatment. Retrieved from https://www.rwlwater.com/anaerobic-digestion/

Wedwitschka, H., Jenson, E., & Liebetrau, J. (2016). Feedstock Characterization and Suitability Assessment for Dry Anaerobic Batch Digestion. *Chemical Engineering & Technology, 39*(4), 665-672. doi:10.1002/ceat.201500413

wettipgasmeter.com. (2018). wettipgasmeter.com: gas meters for laboratories. Retrieved from http://www.wettipgasmeter.com/meters.php

Yadvika, Santosh, Sreekrishnan, T. R., Kohli, S., & Rana, V. (2004). Enhancement of biogas production from solid substrates using different techniques—a review (Vol. 95, pp. 1-10). England: Elsevier Ltd.

Yang, L., Huang, Y., Zhao, M., Huang, Z., Miao, H., Xu, Z., & Ruan, W. (2015). Enhancing biogas generation performance from food wastes by high-solids thermophilic anaerobic digestion: Effect of pH adjustment. *International Biodeterioration & Biodegradation, 105*, 153-159. doi:10.1016/j.ibiod.2015.09.005

Yilmazel, Y. D., & Demirer, G. N. (2013). Nitrogen and phosphorus recovery from anaerobic co-digestion residues of poultry manure and maize silage via struvite precipitation. *Waste Manag Res, 31*(8), 792-804. doi:10.1177/0734242X13492005

Zhang, C., Xiao, G., Peng, L., Su, H., & Tan, T. (2013). The anaerobic co-digestion of food waste and cattle manure. *Bioresour Technol, 129*, 170-176. doi:10.1016/j.biortech.2012.10.138

Zhang, H., & Matsuto, T. (2011). Comparison of mass balance, energy consumption and cost of composting facilities for different types of organic waste. *Waste Management, 31*(3), 416-422. doi:10.1016/j.wasman.2010.09.010

Zhang, Q., Hu, J., & Lee, D.-J. (2016). Biogas from anaerobic digestion processes: Research updates. *Renewable Energy, 98*, 108-119. doi:10.1016/j.renene.2016.02.029

Zhong, Y., Roman, M. B., Zhong, Y. K., Archer, S., Chen, R., Deitz, L., . . . Liao, W. (2015). Using anaerobic digestion of organic wastes to biochemically store solar thermal energy. *Energy, 83*, 638-646. doi:10.1016/j.energy.2015.02.070

The Zoo that Could. *Detroit Zoo.* Retrieved from http://thezoothatcould.org/