Advancing Multi-stage Anaerobic Digestion Technologies through Improved Hydrolysis Processes

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Executive Summary

Anaerobic digestion (AD) is an attractive technology because it can be used to generate energy from the organic fraction of municipal solid waste (OFMSW). AD can be used to convert solid waste material into biogas, or methane. AD is used broadly in the US in the wastewater industry to treat biosolids; however, the wet digestion technologies used for wastewater (complete mix and plug flow reactors) are not directly applicable to high-solids OFMSW. Alternatively, high-solids AD technologies are readily applicable to food waste or OFMSW, and high-solids AD is the technology used in recently built commercial-scale AD facilities (e.g., in San José, California). However, these currently available technologies are not economically viable in most regions. Thus, technology advances are needed.

Multi-stage AD technologies are of particular interest because they can produce more energy per mass of waste. Increased performance is due to use of separate reactors for the waste hydrolysis and methanogenic stages allowing each process to be optimized individually for maximum energy generation. However, multi-stage technologies typically incur higher capital as well as operation and maintenance costs; therefore, further process advancements are needed to reduce costs and increase performance (EPA Biosolids Technology Fact Sheet, 2006; Linville et al., 2015). Significant past work has focused on developing optimized technologies for the methanogenesis stage, but there has been a critical need to improve hydrolysis processes. Thus, the focus of this study was on improving hydrolysis processes for multi-stage AD applicable to OFMSW. The technology advanced in this work uses a high-solids leachate bed reactor operated in batch mode for waste hydrolysis, wherein liquids are percolated over the solids to promote microbially-mediated waste solubilization. The perchlorate, or leachate, is fed to a continuous high-rate methanogenic reactor to produce biogas.

A specific challenge for achieving maximum hydrolysis rates of OFMSW is the presence of inhibitors including ammonia and salinity, which limit microbial processes. Further, these inhibitors can lead to process instability and even process failures compromising economic viability. Elevated levels of ammonia and salinity (>1.7 g total ammonia nitrogen [TAN]/L and 3.5 g Na+/L) are often found in high-solids AD systems that recycle leachate, or percolate, because these inhibitors are often present in feedstocks and build up due to recirculation over long-term operation. However, our past research showed that optimal hydrolysis can be maintained by using specialized microbial inocula that are adapted to elevated salinity and ammonia levels (i.e., acclimated inocula). Thus, this past research suggested that advances in microbial community management (i.e., use of these acclimated inocula at startup and development of methods to maintain desired microbes within leachate beds) could improve process efficiency for high-solids waste hydrolysis processes. Economic analysis was conducted to evaluate the impact of technical solutions. The economic analysis included estimation of one-time capital investments,
revenues from biogas sales, and selected annual operating costs for the high-solids multi-stage technology. Comparisons with existing systems were made only with respect to capital costs due to the lack of availability of appropriate operation and maintenance costs data.

To guide development of microbial management strategies to avoid process upsets and failures due to inhibitors, laboratory-scale studies were conducted. Studies focused on development of suitable methods to maintain stable populations of optimal ammonia- and salt-tolerant microbial communities within reactors during long-term operation (months to years). Fresh batches of waste can be inoculated with pre-digested waste left behind from the previous batch, although robust inoculation methods had not previously been developed for high-solids hydrolysis processes. Such approaches must be able to support optimal performance over long-term operation even as suboptimal conditions, including elevated levels of salinity and/or ammonia, develop. Herein, performance was compared for leach bed reactors (LBRs) seeded with unacclimated or acclimated inoculum (0-60% by mass) at start-up and over long-term operation. Research showed that high quantities of inoculum (~60%) increase waste hydrolysis and are beneficial at start-up or when inhibitors start to be substantially elevated, which may occur after several months of operation. After start-up (~112 days) with high inoculum quantities, leachate recirculation leads to accumulation of specific inhibitor-tolerant hydrolyzing bacteria in leachate. Then, during long-term operation, low inoculum quantities (~10%) effectively increase waste hydrolysis relative to without solids-derived inoculum. Importantly, molecular analyses indicated that combining digested solids with leachate-based inoculum doubles (4.4 x 10^10 vs. 2.1 x10^10 bacteria/g fresh waste) the quantities of bacteria contacting waste over a batch. Additionally, digested solids inoculum provides different microbes than recirculated leachate. Critically, the known cellulose hydrolyzers *Clostridia* were only found at high levels in digestate. By contrast, other known hydrolyzers *Bacteriodes* were predominant in leachate. Thus, combining solids-based and leachate-based inoculation is expected to maximize hydrolysis rates.

To determine if findings regarding the benefits of providing solids-derived inoculum could be extended to large-scale AD processes, demonstration-scale studies were conducted. An improvement in hydrolysis rates as a function of inoculum percentage was not observed. However, at the demonstration-scale ammonia and salinity concentrations were much lower than at the laboratory scale. Conductivity values were typically below 1.0-1.1 mS/cm compared to 45 mS/cm in the laboratory-scale. Similarly, ammonia levels were less than 0.25 g TAN/L at the full-scale compared to 3.5 g TAN/L at the laboratory-scale. At the laboratory-scale, inhibitor levels were artificially elevated to allow us to study the benefits of inoculation when it is most critical, e.g., when conditions are not optimal. The laboratory-scale experiments were designed to be representative of full-scale systems after ammonia and salinity have built up (after months of operation). At the demonstration-scale, reactor start-up was conducted similarly to how it would be done at full-scale; however, over the operational period (6 months) inhibitors had not yet built up. Although ammonia and salinity concentrations were expected to increase over
time in the demonstration-scale system, concentrations may have remained low in this case because operation time was too short to observe significant buildup of these inhibitors, and dilution water was added to account for water losses caused by operating challenges. Operational challenges occurred due to extreme cold weather conditions, which caused some pipe damage and leaks during the course of the demonstration-scale system. These operational challenges due to cold weather are easily addressable in full-scale systems, but impacted results in our experimental set up. Thus, findings suggested that the benefits of inoculation may only be significant when high levels of inhibitors are present, which will depend on operation time and waste characteristics. However, additional large-scale studies with various waste sources would be needed before firm conclusions can be drawn. Such studies could be run for longer term (~over a year) or leachate inhibitor concentrations could be artificially altered.

Economic analysis indicated that the multi-stage technology investigated is competitive with existing technologies on the basis of capital costs for the same MMBtu/yr of biogas generated. Capital costs were comparable for both an existing full-scale low-solids multi-stage system and a high-solids single stage system. Payback periods for this investment were greater than 10 years across all scenarios with baseline energy prices due to low operating profit estimates compared to the capital investment. Payback period analysis considered revenue from power generation, select operating costs, and estimated maintenance costs. For increased energy selling prices ($15.30/ MMBtu sold), payback periods of 4-5 years are possible depending on the scale; this selling price approximately equates to a consumer price of $30.60 MMBtu. Further, analysis suggested that improving hydrolysis rates and therefore reducing solids residence times results in a reduction of capital costs (up to a ~12% reduction for a 14-day solids residence time). Therefore, the economic analysis indicates that advancing hydrolysis technologies will improve overall AD economics. Further advancements are desired to improve profitability when energy prices are low.
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<th>Acronym</th>
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<td>AD</td>
<td>Anaerobic digestion</td>
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<td>BMP</td>
<td>Biochemical methane potential</td>
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<td>LBR</td>
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<td>Multi-stage anaerobic digestion</td>
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<td>mS/cm</td>
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<td>Organic fraction of municipal solid waste</td>
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<td>Organic loading rate</td>
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<td>Programmable logic controller</td>
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Introduction

Existing and proposed regulations requiring organic waste diversion from landfills, as well as public interest, are driving an ever-increasing need for sustainable waste management technologies for handling organic wastes. Multiple states and some cities already have enacted bans on food waste going to landfills (Staley and Kantner, 2015). Organic wastes that are diverted from landfills are often composted; however, composting alone does not harness the renewable energy generation potential of organic wastes, and in many cases there is not a large enough demand for composted solids to justify these programs. Alternatively, anaerobic digestion (AD) can be employed for bioenergy generation, and digestate can be subsequently composted to generate marketable soil amendments.

Further, life cycle assessment demonstrates that AD represents the most environmentally sustainable waste management technology for food waste (Levis and Barlaz 2011). Recently, the city of San José, California has constructed one of the first commercial-scale in-vessel AD facilities for organic fraction municipal solid waste (OFMSW) in the United States (Zero Waste Energy, 2012) and others have been developed in California as well as a few other locations in North America (Ontario, British Columbia and Central Florida). While application of AD to wastewater biosolids is practiced broadly in the US, these wet digestion technologies (complete mix and plug flow reactors) are not directly applicable to high-solids OFMSW; OFMSW must be significantly pre-processed and co-digested with wastewater if wet (low-solids) technologies are used. Alternatively, high-solids AD technologies have been applied to food waste for decades in Europe (Rapport et al. 2008, Mata-Alvarez et al., 2000), and recent commercial-scale AD facilities built (e.g., in San José, California) use high-solids (dry fermentation) technologies. However, AD is not widely applied to commercial food waste or OFMSW in the US because currently available AD technologies are not economically viable in most regions, and landfilling remains an acceptable and economically favorable option of disposal. Thus, advances are needed to improve waste conversion and biogas generation. To support the solid waste industry, efforts should target increasing profitability for high-solids technologies.

Multi-stage AD processes are of particular interest because these technologies utilize separate reactors for the waste hydrolysis and methanogenic stages allowing each process to be optimized individually for maximum energy generation (Bolzonella et al. 2006; Selvam et al. 2010; Voelklein et al. 2016, Xu et al. 2011). In these systems, high-solids waste is placed in leachate bed reactors where water is passed over the waste to promote microbially-mediated solubilization. The water that is percolated over the waste is referred to as leachate. The leachate can then be passed to a high-rate methanogenic reactor. However, separate reactors incur higher capital costs (Ward et al. 2008), and thus, maximizing process efficiency is critical. A substantial body of past research has focused on optimizing methanogenesis processes (Chen et al., 2008; Sung and Liu, 2003); however, research to advance hydrolysis processes for food waste and OFMSW
applications is surprisingly limited. Development of improved hydrolysis processes has been identified by AD industry technology providers as a critical need (personal communications with Harvest Power and GICON).

A specific challenge for achieving maximum hydrolysis rates of food wastes and OFMSW is the presence of inhibitors including ammonia and salinity, which are known to limit microbial processes including both hydrolysis and methanogenesis (Chen et al. 2008; Hashimoto 1986; Angelidaki and Ahring 1993; Angenent et al. 2002; Shahriari et al. 2012; Wilson et al., 2013). Inhibition of AD performance due to elevated ammonia and salinity has been demonstrated to occur at concentrations >1.7 g total ammonia nitrogen (TAN)/L and 3.5 g Na⁺/L, respectively (Chen et al., 2008), and we have recently shown that these inhibitors can substantially impact hydrolysis rates (Wilson et al., 2013). Elevated levels of salinity and ammonia are often found in AD systems that recycle leachate (Chen et al., 2008; Kayhanian, 1994; Shahriari et al., 2012), which can include both wet and dry systems. Leachate recycle is typically employed to help retain methanogens in single-stage AD configurations and to reduce water usage in both single- and multi-stage systems. However, this practice can also lead to build up of inhibitors, which are present in feedstocks or produced as byproducts of digestion (e.g., ammonia). To tackle this challenge, we have developed advanced microbial inocula capable of achieving high rates of hydrolysis even in the presence of elevated ammonia and salinity (Wilson et al., 2013); in batch-scale studies with elevated ammonia and salinity, hydrolysis rates were significantly improved (4- to 10-fold as compared to with standard inocula) using these specifically developed optimized microbial seeds. Further research was needed to develop approaches for establishing and maintaining these optimized microbial populations in large-scale, multi-stage AD systems. The goal of this project was to advance high-solids AD by improving hydrolysis processes through development of an approach to establish and maintain optimal microbial populations in hydrolysis reactors.

The specific project objectives were as follows:

**Objective 1:** Evaluate leach bed reactor operational approaches to control the microbial populations in AD reactors at start-up and over the course of reactor operation via testing both initial seeding methods and process operation to maintain key organisms. This objective focused on determining how hydrolysis rates and methane generation rates can be improved via seeding with pre-acclimated inocula and by optimizing the ratio of fresh waste to digested waste (used as a source of microorganisms for each successive batch of waste). Our experimental approach involved evaluating performance of laboratory-scale reactors for a range of ratios of fresh to digested waste and tracking microbial populations in the solid digestate and leachate. We sought to determine if the operational approaches evaluated would result in maintaining key hydrolyzing organisms and improvements in volatile solids reduction and methane yields.
Objective 2: Develop operational approaches to maintain increased hydrolysis rates during long-term operation. This objective focused on testing inoculum delivery methods for scaled up reactors with a demonstration-scale multi-stage AD system. The inoculum approaches tested included fully mixed digestate inoculation, enhanced leachate inoculation (i.e., introducing post-digestion solid inoculum into the leachate), and simple leachate recirculation. Performance was evaluated by monitoring volatile solids reduction and waste solubilization.

Objective 3: Conduct an economic analysis to evaluate profitability of developed improved hydrolysis processes. This objective focused on conducting the economic analyses needed to guide industry decision making.

Background

Anaerobic digestion is a microbially-mediated process involving three key steps: 1) hydrolysis, 2) acido/acetogenesis, and 3) methanogenesis (Kumar et al. 2011) (Fig. 1). Each step, or process stage, is mediated by a separate group of microorganisms. First, solid waste must be solubilized by hydrolyzing bacteria. Then soluble products are converted to volatile fatty acids (VFAs) and acetate by acidogenic and acetogenic bacteria. Finally, methanogens convert acetate to methane. Additionally, hydrogenotrophic methanogens produce methane from carbon dioxide and hydrogen. Both hydrolysis and methanogenesis can be rate-limiting steps. Hydrolysis is a rate-limiting step for high-solids AD, particularly for the digestion of refractory waste such as lignocellulosic materials (e.g., fruit peels and green wastes) (Nielsen et al. 2004). Achieving optimal methane generation requires that optimal microbial populations are present in AD reactors and that reactor conditions promote maximum activity of each microbial group.

OFMSW of food waste can be challenging to handle via AD because these wastes are complex, variable, and waste components degrade at different rates (e.g. polysaccharides will degrade faster than cellulose). Rapid degradation of some components can result in the build-up of acidic conditions if the methanogenic populations are not able to covert VFAs to methane at a sufficiently rapid rate. Because acid conditions inhibit methanogens, this phenomenon can ultimately result in system failure particularly for single-stage AD systems. Multi-stage AD processes overcome this

![Diagram of microbially-mediated steps involved in conversion of waste materials to methane (CH₄). Both fermentative and oxidative methanogenesis are shown.](image-url)
challenge because each process stage occurs in a separate reactor, so reactor conditions (e.g., pH) can be controlled to prevent failure. Further, in principal, multi-stage AD processes have the potential to produce the highest methane yields because each stage can be optimized independently (Bolzonella et al. 2006; Selvam et al. 2010; Xu et al. 2011) and slow-growing methanogens can be maintained at higher concentrations by using upflow anaerobic sludge blanket reactors or fixed film (FF) reactors for the methanogenesis stage because these reactors retain high quantities of methanogens.

Due to the above-mentioned benefits, our work has focused on the development of multi-stage leach bed-based AD systems. The Colorado State University (CSU) multi-stage anaerobic digestion (MSAD) system uses dedicated hydrolysis reactors, or leach bed reactors (LBRs), to extract organic matter and nutrients. High solids waste substrates are placed in the LBRs where hydrolytic bacteria convert complex organic polymers into more simple organic molecules (Fig. 2). Because more simple organic compounds are much more soluble and mobile than their parent molecules, water passed through the column collects high concentrations of these soluble organic compounds. This water passed through the LBR is thus referred to as leachate. After leachate passes through the LBR, it is stored in the leachate storage tank (LST). The LST serves as an important reaction vessel for the biological and chemical reactions that transform the soluble organic constituents from the LBR into the chemical precursors of bio-methane (i.e., for acido/acetogenesis). From the LST, the leachate is recycled through the LBRs and the methanogenic reactor, or the fixed film (FF) reactor. Methanogenic bacteria colonize the surface area of packing material to form an attached film of microorganisms within the FF reactor. These microorganisms act as a biological filter for the leachate and serve to convert the organic molecules within the leachate into methane biogas. The newly “filtered” leachate is then passed back into the composting tank (LST) to be cycled through the process again.
One benefit of the MSAD process is that high solids waste (>30% solids) can be placed directly in the LBRs with minimal pre-processing. Contaminating materials (e.g., inert solids) do not negatively impact biogas production because they remain in the LBRs and can be removed with the digestate at the end of each batch. Furthermore, because it is a high solids process, high waste loading rates can be achieved (Rivard 1993). Leachate recirculation reduces water input, and therefore also energy input. A preliminary economic analysis for application of this process to OFMSW showed this technology is cost-competitive with composting; however, further improvements to hydrolysis processes were needed to reduce capital costs and increase revenues.

One key challenge was the need to develop suitable methods to maintain stable populations of optimal ammonia- and salt-tolerant microbial communities within reactors during long-term operation. The leach bed based systems used for hydrolysis are operated in batch mode and digested solids are removed at the end of each batch. Past research has shown that hydrolyzing bacteria is often associated with solids (Adney et al. 1991; Zverlov and Schwarz 2008). Therefore, many hydrolyzing bacteria would typically be removed at the end of each batch. Given that days to weeks are required for development of inhibitor-tolerant microbial communities, this standard practice would lead to unstable populations (Wilson et al., 2016). An alternative is that fresh batches of waste could be inoculated with pre-digested waste left behind from the previous batch. However, studies on how microbial seeding methods used in AD reactor operation affect populations of hydrolyzing bacteria are lacking. Thus, research was needed to develop optimal reactor seeding approaches specifically for food waste hydrolysis. Such
approaches must be robust and able to support optimal performance over long-term operation even as suboptimal conditions, including when elevated levels of salinity and/or ammonia, develop. Additionally, optimal seeding methods can be used to reduce reactor start-up times (Liu et al. 2002), which is critical because delays in methane generation can cause issues with project financing. Economics analysis of seeding methods was needed to guide industry decision-making.
Results and Discussion

Laboratory-Scale Study

Laboratory-scale experiments were run to address Objective 1. Experiments were designed to evaluate leach bed reactor operational approaches to control the microbial populations in AD reactors over the course of reactor operation. Our experimental approach was to first test seeding with a range of different ratios of unacclimated or acclimated inoculum to fresh waste (0-60% by mass) to determine how hydrolysis rates and methane generation can be improved at start-up, or when salinity and ammonia concentrations start showing substantial increases. Results showed that volatile solids reduction was higher for 10% and 60% inoculum compared to no inoculum. Next we explored impacts of seeding methods over long-term operation. Retention of digestate, at a range of different ratios of digestate to fresh waste, was tested in a fully operational, laboratory-scale multi-stage AD system run for over 192 days. During long-term operation, low inoculum quantities (~10%) were found to effectively increase waste hydrolysis relative to no inoculum. Molecular biology analyses suggested inhibitor tolerant hydrolyzing bacteria built up in the leachate by day 112. Thus, results showed that key hydrolyzing bacteria can be maintained when digestate is retained and leachate is recirculated, and these practices ultimately increase methane production.

The laboratory-scale study has been published in the peer-reviewed journal *Bioresource Technology* (Wilson and De Long, 2016). Readers are also referred to that publication.

Reactor configurations

Two different configurations were used in this study to investigate the effects of inoculum percentage at start-up and over time. For experiments focused on reactor start-up, 4 LBRs were connected to individual LSTs without connections to methanogenic reactors (Fig. 3 a, d). Utilizing separate LSTs allowed for isolation of the effects of inoculum percentages without the influence of microbial community development from the other LBRs or methanogenic reactors. In full-scale systems that incorporate recycle, it can take many batches (~months of operation) for inhibitors to build up in leachate. Thus, ammonia and salinity were artificially elevated to 3.5 g TAN/L and 45 mS/cm to reduce experiment times and allow us to investigate inoculation methods under the most challenging conditions.
Figure 3. Reactor configuration for determining performance at start-up (a). Plan view of LBR depicting spacing of leachate distribution ports (b). Reactor configuration for determining performance over time (c). Configurations include leach bed reactors (LBRs), leachate storage tank (LST), and a fixed film reactor (FF). Arrows depict leachate flow path. LBR schematic depicting layered inoculum and fresh waste (d).

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For long-term operation, a multi-stage system was utilized that contained a triplicate set of LBRs, 1 LST, and 1 FF reactor (Fig. 3c). Effluent from the FF was pumped back to the LST. Leachate also was pumped from the LST to the top of the LBRs as described in Materials and Methods. The FF was filled with plastic packing material (Bioprotz moving media, Entex Technologies). Leachate samples for liquid analysis were collected from the LST and FF reactor effluent (post-treatment). Samples were collected from the LST, rather than LBR effluent directly, as a means of monitoring pH and VFA concentrations in the composited leachate delivered to the LBRs and FF to ensure conditions were not inhibitory (e.g., >6.5 pH in the LST) (Ahn et al., 2010).

Impact of inoculation on waste hydrolysis at start-up

We expected waste reduction would be limited by the number of organisms provided as inoculum in the LBRs under elevated ammonia and salinity conditions. As expected, results demonstrated that higher percentages of inoculum are beneficial and improve volatile solids (VS) reduction. Initially, with acclimated inoculum at 60% and a digestion period of 16 days, reactors demonstrated an average VS reduction of 69.5% (±3.5) (Fig. 4).
Figure 4. VS reduction as a function of percentage of inoculum at start-up. At start-up, reactors were seeded with acclimated, unacclimated or no inoculum operating under elevated ammonia and salinity. Three batches (starting on days 0, 16, and 32) were run for each percentage. Multiple data points at each percentage demonstrate results for successive batches.

Results from our study demonstrated that hydrolysis performance was better than or comparable to previously reported performances for dry digesters operating under lower ammonia and salinity, despite the elevated levels present. For example, Xu et al. (2014) achieved 45.4 - 69.4% VS reduction in LBRs treating food waste inoculated with wastewater treatment plant (WWTP) AD sludge with a 16-day residence time. Similarly, Yan et al. (2014) demonstrated 58.7 – 68.1% VS reduction in LBRs fed simulated food waste and inoculated with AD sludge and cow manure. Lu et al. (2008) demonstrated 63% VS reduction in LBRs connected to a UASB with leachate recirculation treating vegetable and flower waste over a 10 day period. Thus, results indicated that high solids conversion can be achieved even in the presence of inhibitors, if required organisms are provided in sufficient quantities via inoculation.

For lower acclimated inoculum percentages, average VS reduction was reduced ~1.5- and 2-fold (10% and 0%, respectively) (Fig. 4). An ANOVA test on triplicate batches at each inoculum percentage indicated that significant differences exist among means for VS reduction (p-value = 0.0001). The significant reduction in solids removal with reduced inoculum percentages demonstrates that the quantity of hydrolyzing bacteria present in LBRs limits performance. VS reduction improved by the end of batch 3 for all inoculum percentages (with VS reduction increasing to 73% for the 60% acclimated inoculum). Performance increases for successive batches likely indicate that the concentration of inhibitor-tolerant hydrolyzing bacteria increased in the systems (e.g., in the digestate). However, VS reduction with 0% inoculum was still 1.9-fold lower than VS reduction with 60% inoculum for the third batch. In the only other study to investigate the impact of inoculum percentage at start-up in LBRs, Xu et al. (2012) also observed a decrease in waste hydrolysis with reduced inoculum percentages (80%, 20%, 5%, and 0%), albeit a less dramatic trend than observed in our study. The less dramatic decrease was likely
because Xu et al. (2012) investigated low ammonia and salinity conditions. Specifically, their study demonstrated 1.13- and 1.14-fold lower VS removals for inoculum percentages of 20% and 5%, respectively, compared to an inoculum percentage of 80%. Xu et al. (2012) recommended a 20% inoculum; however, the study did not test multiple batches over time or 10% inoculum. Ultimately, the substantial reduction in VS removal observed in our study at lower inoculum percentages suggests that higher volumes of acclimated inoculum are needed at start-up, or when salinity and ammonia are observed to increase in leachate, for optimal performance.

To determine if acclimated inoculum enhanced performance compared to unacclimated inoculum, both inocula were tested in parallel at 60%. Interestingly, acclimated inoculum initially demonstrated 68% VS reduction, compared to 56% VS reduction for unacclimated inoculum (Fig. 4). However, both reactors performed similarly by the end of batch 3. Thus, results suggest that inhibitor-tolerant organisms accumulated in as little as 3 batches when a high quantity of inoculum was used (60%). Several studies have demonstrated that acclimated inoculum performs better than unacclimated inoculum in wet systems (Calli et al., 2005; Chen et al., 2008; Omil et al., 1995; Sung & Liu, 2003). For example, we previously demonstrated that an AD sludge-derived inoculum acclimated to elevated ammonia (5 g TAN/L) fed manure and food waste performed significantly better than unacclimated sludge directly exposed to elevated ammonia concentrations in wet batch reactors (Wilson et al., 2013). Thus, results herein extend this finding to LBRs and provide guidance on the quantity of inoculum needed to maximize solids reduction at start-up (~40-60%) or when inhibitors build up.

**Impact of inoculation on system performance over long-term operation**

To determine how inoculum percentages and connection to a FF affect maintenance of desired organisms and performance over long-term operation, experiments were conducted with multi-stage reactors that incorporated leachate recycle for over 190 days. Hydrolysis performance and methane generation were monitored across a range of inoculum percentages (60% - 0%). Average VS reduction for LBRs with low ammonia and salinity at 60% inoculum was slightly better (1.17-fold) than for LBRs with high ammonia (~3.5 g TAN/L) and salinity (45 mS/cm) (Fig. 5). This slight inhibition is consistent with the observations of others (Chen et al., 2008); however, in our study inhibition likely was minimized via the usage of acclimated inoculum. In one of the first studies to determine the effects of elevated ammonia, Kayhanian (1994) demonstrated that high solids digesters performed best at TAN concentrations in the range of 0.6-1 g/L. More recently, Duan et al. (2012) reported significant inhibition at 3 - 4 g TAN/L in high solids AD of sewage sludge with the highest VS reduction reaching only 40% (compared to ~60% for uninhibited reactors) and volatile fatty acid (VFA) concentrations exceeding 10 g/L (Song et al., 2004). In contrast, our study demonstrated VS reduction levels up to an average of 62 (±7)% at 60% inoculum under elevated ammonia and salinity, indicating a benefit for use of inoculums acclimated to elevated ammonia and salinity.
Figure 5. VS reduction as a function of percentage of inoculum over time. Over time, reactors were seeded with unacclimated inoculum under low ammonia to demonstrate optimal reactor performance without inhibition or acclimated inoculum under elevated ammonia and salinity. Multiple data points at each percentage demonstrate results for successive batches. Data points and error bars represent average performance and standard deviation for the set of triplicate reactors for a single batch. Days of operation are indicated at the top of the figure.

Interestingly, in contrast to start-up, over time VS reduction remained relatively constant for decreasing inoculum percentages (Fig. 5). At 60% initial inoculum, VS reduction averaged 62 (±7)% over the first two batches. When inoculum percentages were then decreased to 40% and 20%, VS reduction appeared to decrease slightly. Unexpectedly, VS reduction increased to an average of 67 (±7)% when the inoculum percentage was lowered further to 10%, which was the highest VS reduction demonstrated for any of the percentages. Moreover, this performance at 10% inoculum is comparable to performance demonstrated in reactors operating under low ammonia and salinity.

However, at 0% inoculum, VS reduction once again decreased and demonstrated the lowest reductions of any inoculum percentages. Despite a decreasing trend for VS reduction as a function of inoculum percentage (with the exception of 10% inoculum), the slope of the linear regression for all percentages with acclimated inoculum was not significantly different from zero, suggesting that inoculum percentage did not substantially affect VS reduction over time. However, an ANOVA test revealed significant differences in mean VS reductions among the various inoculum percentages (p-value = 0.0074), and Tukey’s HSD test identified significant differences between the mean VS reduction at 10% and 0% inoculum (p-value = 0.003). No other significant differences between mean VS reductions were observed. Thus, regardless of inoculum percentage, over time reactors demonstrated VS reduction comparable to systems running under
optimal conditions despite the elevated ammonia and salinity levels (Lu et al., 2008; Xu et al., 2014; Yan et al., 2014).

Consistent with solids reduction data, VFA concentrations demonstrated steady performance for decreasing inoculum percentages over time (Fig. 6). At the start of a new batch, leachate VFA concentrations spiked in the LST due to the influx of fresh waste but gradually reduced as readily hydrolysable substrates were converted to soluble products and methane. Steady, low VFA concentrations in FF effluent demonstrated the FF was effective despite elevated inhibitor levels.

![Figure 6](image6.png)

**Figure 6.** VFA concentrations over long-term operation for multi-stage reactors operating under elevated ammonia and salinity (~3.5 g TAN/L and 45 mS/cm). Data are shown for a range of acclimated inoculum percentages over time. Inoculum percentages are noted at the top of each graph section.

pH values ranged from 6.6 – 8.3 and 7.8 - 8.5 in the LST and FF effluent, respectively, over the course of the experiment (Fig. 7).

![Figure 7](image7.png)

**Figure 7.** pH over long-term operation for multi-stage reactors operating under elevated ammonia and salinity (~3.5 g TAN/L and 45 mS/cm). Data are shown for a range of acclimated inoculum percentages over time. Inoculum percentages are noted at the top of each graph section.

Overall, our reactors demonstrated comparable or higher methane generation to previous literature values for reactors operating under low ammonia and salinity conditions (Fig. 8 & Table 1). The highest average methane yield, observed with the greatest inoculum percentage, was 0.260 [± 0.01] L CH₄/ g VSfresh waste (Fig. 8). By
contrast, lower methane production (0.182 L CH₄/ g VS) has been demonstrated in LBRs coupled with a upflow anaerobic sludge blanket fed simulated food waste for a 17-day digestion period at start-up (Xu et al., 2011). Additionally, Han et al. (2002) observed 0.27 L CH₄/ g VS for sequential batch, two-phase anaerobic composting systems fed food waste (with impurities removed) and inoculated with rumen microorganisms with a 10 day solids retention time. Dearman et al. (2007) demonstrated similar or slightly lower methane yields (0.214, 0.229 L CH₄/ g VS) for dry food waste digesters using 10% (w/w) inoculum despite an extremely long digestion period (73 days). Solids reduction is strongly impacted by feedstock composition, and thus lower rates reported by others may also be due, in part, to use of more recalcitrant feedstocks. In the case of Han et al., antimicrobial agents may have been present in the rumen used as inoculum and caused microbial inhibition. However, superior microbial populations likely improved methane production herein.

Interestingly, despite remaining within the range of reported methane yields, average yields in our study decreased with decreasing inoculum at 40% and 20% (0.212 [±0.01] and 0.195 [±0.01] L CH₄/ g VSfresh waste, respectively), but then increased at 10% inoculum and decreased at 0% inoculum, consistent with solids reduction data (Fig. 8). At 10% inoculum, methane yield was 0.230 (±0.02) L CH₄/ g VSfresh waste, and at 0% inoculum yield was 0.207 [±0.01] L CH₄/ g VSfresh waste.

Figure 8. Methane yield over long-term operation for multi-stage reactors operating under elevated ammonia and salinity (~3.5 g TAN/L and 45 mS/cm). Data are shown for a range of acclimated inoculum percentages over time. Inoculum percentages are noted at the top of each graph section.

Table 1. Summary of long-term hydrolysis and methanogenesis performance

<table>
<thead>
<tr>
<th>Inoculum (%)</th>
<th>VS Reduction</th>
<th>Methane Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

18
Thus, collectively our results suggest that after an initial start-up period, low quantities of solid inoculum (~10%) are sufficient under elevated ammonia and salinity (Table 1), and importantly, that even after start-up, solid-phase inoculum addition improves performance relative to no inoculum. The increase in performance observed at 10% was unexpected considering the declining trend for higher percentages and the idea that hydrolyzers are solids-associated (Wang et al., 2010; Zhang et al., 2007). These results led us to hypothesize that desired microorganisms built up in the leachate over time and thus leachate recirculation could also provide a source of hydrolyzing bacteria; recirculation can then increase bacterial contact with the waste substrate. Similarly, others have speculated that leachate recirculation enhances performance based on results from a high-solids, single-stage AD system fed cattle waste operated at 40ºC and 50ºC (El-Mashad et al., 2006). To test this hypothesis and develop a mechanistic understanding of microbial community dynamics in multi-stage AD systems, bacterial communities were characterized over long term operation.

### Quantification of bacteria in the leachate and solids inoculum

We sought to determine if leachate recirculation could provide a source of hydrolyzing bacteria and first quantified bacteria in the leachate and solid waste inoculum to determine how the quantities of bacteria present compared. Quantitative polymerase chain reaction (qPCR) test data revealed 60% inoculum resulted in the addition of ~3.1x10^{11} bacteria/ g fresh waste to the LBR (without including bacteria contributed by leachate), while 10% inoculum provided ~2.3x10^{10} bacteria/ g fresh waste (Table 2). Similarly, the total volume of leachate recirculated through the LBR over a 16-day batch period was found to provide ~2.1x10^{10} bacteria/ g fresh waste; this value was calculated based on a leachate sample collection on day 192.

**Table 2. Estimated quantity of bacteria in inoculum**
<table>
<thead>
<tr>
<th>Inoculum (%)</th>
<th>Bacteria/ g fresh waste (standard deviation)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>$3.1 \times 10^{11}$ ($2.5 \times 10^{11}$)</td>
</tr>
<tr>
<td>40</td>
<td>$1.4 \times 10^{11}$ ($1.1 \times 10^{11}$)</td>
</tr>
<tr>
<td>20</td>
<td>$5.1 \times 10^{10}$ ($4.2 \times 10^{10}$)</td>
</tr>
<tr>
<td>10</td>
<td>$2.3 \times 10^{10}$ ($1.9 \times 10^{10}$)</td>
</tr>
</tbody>
</table>

*Bacterial quantities were estimated based on measurement of bacterial 16S rRNA genes per g of fresh waste added to leach beds via acclimated inoculum. The assay utilized in our study only targets bacteria and avoids methanogen detection. For simplicity, we assumed that all bacteria contained one copy of the 16S rRNA gene, although in reality bacteria can contain between 1 and 10 copies of this gene. Inoculum analyzed was collected on day 192. Thus, for all of the inoculum percentages (10-60%), bacterial 16S rRNA gene quantities were estimated based on a single sample to eliminate the impact of changes over time. The amount of fresh waste added to each reactor remained constant over time, and the amount of inoculum was adjusted accordingly. Standard deviations are for qPCR reactions run on DNA extracted from quintuplicate samples.

These data do not indicate the type or activity of bacteria, but results support the hypothesis that leachate is a good source of inoculum for hydrolysis after sufficient time has allowed microorganisms to build up in the leachate. Notable improvements in performance were not seen until day 112, so a high percentage of inoculum may be beneficial initially for at least 2-4 batches. The initial percentage of inoculum used likely will affect the number of batches required to establish sufficient hydrolyzing populations because high initial volumes of inoculum will add greater quantities of microorganisms to the system. The inoculum percentage could also be decreased slowly after 2 batches as was done here to continue building up desired organisms, and simultaneously avoid system overload by slowly introducing higher quantities of OFMSW. Further work is necessary to clarify the most advantageous method of decreasing inoculum over time. However, within ~3-4 months, results suggest that low volumes of inoculum may be utilized consistently. Importantly, molecular data also indicate that addition of 10% inoculum doubles the quantity bacteria that contact fresh waste over the course of a batch; this finding is consistent with the superior performance observed at 10% inoculum compared to leachate alone.

**Changes in microbial community composition over time**

Because the type, as well as the quantity, of bacteria control performance, we sought to determine if microbial community compositions between the solid inoculum and leachate after 192 days of operation were similar. Thus, we examined the total bacterial, fermenting, and hydrolyzing microbial communities among fresh waste, leachate, and inoculum. Additionally, digested samples were analyzed after a 5-day digestion period to determine microbes present at the early stages of a batch. Over time (weeks to months of operation) microbial communities in leachate became similar to microbial communities in digestate (Fig. 9), while the microbial communities in the feedstock remained distinct. Similarly, Staley et al. (2012) demonstrated distinct shifts in microbial community composition in decomposed refuse compared to fresh refuse in landfills.
Initially, through day 80, leachate bacterial communities were distinct from other communities. However, by day 112, the communities had undergone a large shift, and by day 192, the leachate communities clearly clustered with inoculum and digested waste communities (Fig. 9). Similar results were observed for hydrolyzing bacteria, even though hydrolyzers have been considered solids-associated (not shown). Thus, molecular data suggests that recirculated leachate can indeed be a useful inoculum for hydrolysis after a lag period if the leachate microbial communities are properly developed. Interestingly, in one of the few studies to investigate microbial community changes in AD leachate, Degueurce et al. (2016) concluded there was little transfer of microorganisms between leachate and solid waste in LBRs digesting manure, and, instead, microorganisms inherently present in the manure strongly impacted reactor performance. The contrasting results observed in our study may be explained by the difference in feedstocks; manure often is used as an inoculum due to its high quantity of microorganisms, which likely dominated reactor communities, whereas OFMSW microorganisms were readily outcompeted by optimized inoculum microbes. The OFMSW feedstocks likely did not contain high levels of bacteria tolerant of elevated salinity and ammonia. Additionally, lower redox in the AD system likely contributed to selection of different microbes despite storage of the food waste in large bins that may have become anaerobic.

Results also indicated that the inoculum delivery approach impacted development of desired microbial communities within fresh waste during the digestion process. Delivery via layering inoculum (LI) and mixing inoculum (MI) were compared to no inoculum (NI). For LI, original inoculum and 5-day digested waste formed a tight cluster when compared to fresh waste and early leachate, indicating relatively successful transfer of inoculum.
microbes to the fresh waste (Fig. 9). However, inoculum and fresh waste layers were distinguishable from each other (Fig. 9). For MI, the microbial community composition grouped generally between the LI waste and LI inoculum layers; these results suggest that thorough mixing is likely beneficial. Interestingly, the NI microbial communities generally clustered with the LI waste layer and MI, indicating that after 192 days, leachate delivered generally the same types of bacteria to the fresh waste as the solid waste inoculum. However, NI communities grouped closer to the LI fresh waste layer than the inoculum layer, consistent with the observed benefits of inoculation with digested waste. These results are similar to previous research indicating that surface-attached bacteria are important for hydrolysis, particularly for cellulose (Lu et al., 2008; Wang et al., 2010). In one of the few studies to look at inoculation methods, El-Mashad et al. (2006) demonstrated that adding inoculum throughout the reactor height compared to leaving previously digested waste at the bottom of reactors increased methane generation. Thus, enhanced delivery methods (e.g., mixing) may increase hydrolysis performance further. Future research is needed to develop enhanced inoculation approaches suitable for full-scale implementation in dry digestion technologies.

Identification of inhibitor-tolerant microorganisms

Sequencing results revealed distinct types of microbes present in inoculum, leachate, and fresh waste (Table 3).

<table>
<thead>
<tr>
<th></th>
<th>Fresh Waste</th>
<th>Inoculum</th>
<th>Leachate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aminobacterium</strong></td>
<td>0.0</td>
<td>3.5</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Other Clostridiales</strong></td>
<td>0.8</td>
<td>21.5</td>
<td>3.0</td>
</tr>
<tr>
<td><strong>Bacteroides</strong></td>
<td>0.0</td>
<td>28.6</td>
<td>66.8</td>
</tr>
<tr>
<td><strong>Unknown Bacteria</strong></td>
<td>0.0</td>
<td>0.0</td>
<td>2.7</td>
</tr>
<tr>
<td><strong>Proteiniphilum</strong></td>
<td>0.0</td>
<td>0.0</td>
<td>3.4</td>
</tr>
<tr>
<td><strong>Mollicutes</strong></td>
<td>0.0</td>
<td>0.0</td>
<td>3.6</td>
</tr>
<tr>
<td><strong>Streptococcus</strong></td>
<td>3.3</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Lactococcus</strong></td>
<td>14.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Leuconostac</strong></td>
<td>16.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Weissella</strong></td>
<td>23.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Lactobacillus</strong></td>
<td>32.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Other Bacteria</strong></td>
<td>10.1</td>
<td>28.1</td>
<td>20.4</td>
</tr>
<tr>
<td><strong>Family XI</strong></td>
<td>0.0</td>
<td>8.9</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Unclassified Clostridiales</strong></td>
<td>0.0</td>
<td>9.4</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*Identifications were based on Illumina MiSeq sequencing of duplicate samples. Higher taxonomic levels are given when genus-level identification was not possible. The label identified as “Other” indicates grouped phylotypes that individually comprised less than 5% abundance. Two representative samples each of inoculum, leachate, and fresh waste were chosen for analysis. Leachate and inoculum analyzed were collected on day 192.

Interestingly, the microbial community in fresh waste was dominated by anaerobes, as well as microorganisms associated with food waste. *Lactobacillales* accounted for over 89% of the microorganisms in the fresh waste, although its presence was less than 1 and
7% abundance in the leachate and inoculum, respectively. Within this order, 4 main genera (*Leuconostoc, Lactococcus, Weissella, and Lactobacillus*) were identified (Table 3), all of which are acid-tolerant anaerobes largely used in the fermentation of dairy products and vegetables. The dominance of these anaerobes was somewhat unexpected and could reflect the particular waste utilized in this study or storage method of food waste prior to collection.

Although microbial communities in the leachate and inoculum were similar, differences observed suggest complimentary capabilities. *Bacteroides* was the dominant genus in leachate and comprised over 25% of the solid waste inoculum. *Bacteroides* are known to be cellulolytic and carbohydrate-utilizing (Wang et al., 2010). The particular species (*Bacteroides coproesuis*) observed in our reactors was first isolated in 2005 from a swine-manure storage pit (Whitehead et al., 2005) and has been documented since then in digesters treating municipal sludge and waste from cattle farms. Results indicated that hydrolyzing bacteria (*Bacteroides*) are present in the leachate. Based on these observations, *Bacteroides* are likely ammonia and salinity tolerant and contribute to improved performance under these conditions. *Clostridiales*, bacteria well known for their cellulolytic capabilities (Wang et al., 2010), comprised 40% of the inoculum communities (including Family XI, unclassified *Clostridiales*, and other *Clostridiales*), but were present at very low levels in the leachate (3%) and fresh waste (<1%). This observation is consistent with previous studies indicating *Clostridium* are solids-associated (Lu et al., 2008; Wang et al., 2010) and may help explain observed performance improvements when solid inoculum was provided. Thus, combining leachate recirculation with inoculation via digested solids provides high levels of two key types of hydrolyzing (cellulose-degrading) bacteria: *Bacteroides* and *Clostridium*.

**Conclusions based on laboratory-scale experiments**

The challenges associated with inhibitory ammonia and salinity concentrations encountered in AD systems can be overcome via hydrolysis seeding methods. Inoculum should be established by maintaining high levels of digested waste (~40-60% by mass) in hydrolysis reactors to seed successive batches at start-up, or perhaps more importantly, when salinity and ammonia are observed to be elevating. After a lag period, desired inhibitor-tolerant hydrolyzers accumulate in recirculated leachate. Thus, leachate recirculation combined with digested-waste inoculum (~10% by mass) emerged as the best option for improved performance based on laboratory-scale data. Additional experiments were conducted to determine if these conclusions could be extended to larger scale systems.

**Demonstration-scale Experiments**
Based on positive findings for Objective 1, we sought to determine if solids and leachate based inoculation strategies would be successful in scaled up systems. Thus, demonstration-scale experiments were run to address Objective 2. Experiments were designed to test inoculum delivery methods. Results of the laboratory-scale experiments indicated that a small amount of digestate could provide a large amount of additional bacteria (e.g., 10% digestate by mass added doubled the number of bacteria available for waste conversion). However, new inoculum delivery methods were needed that were applicable to high-solids AD systems at large scale. Thus, the focus of the subsequent demonstration-scale experiments was to test various inoculum delivery methods in a substantially larger scale multi-stage anaerobic digester system (with 60 gal LBRs, see Materials and Methods). Importantly, for these demonstration-scale experiments, salinity and ammonia were not artificially elevated. Instead salinity and ammonia levels were allowed to build up naturally, with dilution water only added to refill the Leachate Storage Tank as needed, due to losses from leaks and any evaporation. After several months of operation, inhibitor levels were still substantially lower than those used in the laboratory-scale experiments. Although ammonia and salinity concentrations were expected to increase over time in the demonstration-scale system, concentrations may have remained low in this case because operation time was too short to observe significant buildup of these inhibitors and water was added to account for losses caused by operational challenges. Operational challenges occurred due to extreme cold weather conditions, which caused some pipe damage and leaks during the course of the demonstration-scale experiments. These operational challenges due to cold weather are easily addressable in full-scale systems, but impacted results in our experimental set up.

These experiments focused on comparing three main methodologies of inoculation: fully mixed digestate inoculation, enhanced leachate inoculation, and simple leachate recirculation. Due to the relative performance of the 10% and 0% digestate controls in this and previous studies, it was determined that there could be opportunities in using leachate as a primary inoculum transport mechanism. In the enhanced leachate inoculation method, the post-digestion solid inoculum was introduced into the leachate in an attempt to increase the concentration of critical hydrolyzers in the leachate (i.e., to enhance the leachate). Two methods of enhanced leachate inoculation were investigated (injection and top). These approaches were explored to reduce costs associated with inoculation. Performance was evaluated by monitoring volatile solids reduction and waste solubilization, via COD concentrations in the leachate.

**Experiment setup**

Six experimental batches were conducted in the demonstration-scale MSAD. Three different inoculation procedures were evaluated in these experiments including mixed, injected, and top inoculated, as compared to simple leachate recirculation (Table 4). The most commonly used method was the mixed inoculum method. In this configuration, the inoculum was manually mixed into each LBR batch prior to being placed in the LBR at the beginning of each run. This process was conducted by placing the food waste substrate in
an even layer on a plastic tarpaulin. Then the appropriate mass of inoculum was placed on the top of the substrate, and the entire pile was thoroughly mixed before placing it into the LBR. The inoculation method was the same regardless of the specific inoculation ratio (60% or 10%). Two methods of enhanced leachate inoculation were investigated (injection and top in Table 4). The first method (injection; evaluated exclusively in experimental batch 4) utilized a leachate/digestate contact vessel to transfer microbes to the leachate before being delivered to the LBR. The resulting leachate was then sprayed onto the un-inoculated substrate. The second method (top; Table 4) was developed with the goal to simplify the enhanced leachate methodology. In this method, digestate was added as a discrete layer on top of a simple filter placed on top of the un-inoculated substrate within the LBR. This method used the action of leachate percolation through the digestate layer to carry the inoculum throughout the LBR. More details on the inoculation methods are included in the Inoculation Addition Section (see Materials and Methods).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dates of Operation</th>
<th>Inoculation Ratios (% by wet mass)</th>
<th>Inoculation Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1</td>
<td>08/24/2015 – 09/14/2015</td>
<td>60%</td>
<td>Mixed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>Mixed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td>Batch 2</td>
<td>09/15/2015 – 10/5/2015</td>
<td>60%</td>
<td>Mixed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>Mixed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td>Batch 3</td>
<td>10/5/2015 – 11/05/2015</td>
<td>60%</td>
<td>Mixed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>Mixed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td>Batch 4</td>
<td>11/09/2015 – 12/07/2015</td>
<td>10%</td>
<td>Injection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10%</td>
<td>Mixed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td>Batch 5</td>
<td>01/15/2016 – 02/22/2016</td>
<td>10%</td>
<td>Top</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0%</td>
<td>-</td>
</tr>
</tbody>
</table>

Experiments 1-4
For experimental batches 1-4, solids data was the primary analysis for performance. For these experiments, it was important to determine how the various inoculum additions (i.e. 10 % versus 60 %) and approaches impacted the total solids (TS) and VS destruction of the food waste. The % VS at the beginning and end of each batch was measured, and the difference in the %VS values was calculated to determine a change in %VS. As each experimental batch included a non-inoculated control, the decrease in %VS over the non-inoculated control was calculated for each batch. This method of comparing LBR performance was found to be limited in its application due to leaching of fixed and
volatile solids from the solids mass into the leachate. It is important to note that the decrease in % VS reported for Experiments 1-4 is different from the VS reduction (%)/\% \textit{VS} \text{reduction} reported for the laboratory-scale studies and Experiment 5, and these values should not be compared.

Changes in %VS were found to be relatively similar across all inoculum additions tested (Fig. 10). The maximum improvement over the control was near 5 %, while the maximum decrease in reduction of %VS was near 5 %. There was no evidence that inoculated batches performed substantially better than non-inoculated controls. However, results for the decrease in % VS were lower than expected (see discussion below). Thus, it is possible we were not able to detect a benefit of inoculation in batches 1-4 due to overall suboptimal VS removal. Additionally, these demonstration-scale reactors were operated under lower ammonia and salinity than the laboratory-scale reactors. Under stressed conditions, one could expect that inoculums would show a benefit in comparison to non-inoculated controls.

![Figure 10](image)

**Figure 10.** Experimental batches 1-4 (B1-B4) with the % improvement for reduction of %VS of the inoculated batches compared to the non-inoculated control for that batch of experiments. Calculations are described in Materials and Methods.

<table>
<thead>
<tr>
<th>Table 5: VS Reductions Measured in Experimental Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1-60% Inoculum Mixed</td>
</tr>
<tr>
<td>Inoculum Type</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>B1-10% Inoculum Mixed</td>
</tr>
<tr>
<td>B2-10% Inoculum Mixed</td>
</tr>
<tr>
<td>B3-60% Inoculum Mixed</td>
</tr>
<tr>
<td>B3-10% Inoculum Mixed</td>
</tr>
<tr>
<td>B4-10% Inoculum Injection</td>
</tr>
<tr>
<td>B4-10% Inoculum Mixed</td>
</tr>
</tbody>
</table>

Of note is that the decrease in % VS over the process ranged from 1% to 15 % (Table 5). Pre-digestion %VS values for these experimental batches started at an average value of 88.8 ± 5.2 % VS, and the post-digestion VS values averaged 82.0 ± 13.3 %. Resulting average decreases were 6.9 ± 4.7 % VS. These decreases in % VS were lower than expected, and the final VS content of the processed material was higher than expected. By contrast, the % VS decrease observed in the laboratory-scale experiments was substantially higher (average decrease of 19.8 ± 6.1%; total VS reduction of > 49%). Experimental batches 1-4 were conducted under largely suboptimal conditions. During these tests, multiple supporting systems within the demonstration-scale MSAD were experiencing technical issues. Experimental batches 1-3 were conducted while the pump controllers were not operating in a reliable way. As such, leachate would stop being delivered to the LBRs for as many as 16-48 hours during the test. As leachate delivery was the primary method by which heat was transferred to the LBR, the temperatures would fluctuate and occasionally drop to as low as 15°C. The weather conditions during experimental batch 4 (and the first weeks of experimental batch 5) overwhelmed the heating system in the demonstration unit and the temperatures at the outlet of the LBR fell as low as 25°C and only rose to 32°C as a high temperature. These variable and overall low-temperature conditions during the bulk of experimental batches 1-4 undoubtedly impacted digestion rates in the process, resulting in the lower than typical biodegradation in the process. Despite these operational issues, the process clearly resulted in substrate degradation (as observed by decrease in %VS) and comparisons amongst inoculum amounts and approaches could still be made as shown in Fig. 10. Further, in experimental batches 1-4 the measured pH values remained in a narrow range between pH 7.5 and 7.75 without the need for pH adjustment. This indicated that the methanogens in the system were operating at a rate high enough to limit organic acid build up.

**Experiment 5 Results**

After the first four experimental batches, we focused on developing technical solutions to the operational problems. This was largely successful, and although it took around 6 days to heat the mass of each LBR to a minimum temperature of 32°C, the process was maintained between 32°C and 35°C for the duration of experimental batch 5.

Due to limitations in data collected for experimental batches 1-4, for experimental batch 5, the quantity of the entire mass of the TS and VS at the beginning and the end of the
experiment was calculated by multiplying the initial and final TS and VS percentages by the total mass of the substrate (i.e. wet mass; \( m_{\text{wet}} \)) in the reactor at the respective sampling points (see Materials and Methods, Analytical Methods). Thus, for experiment 5, the VS reduction (%) was determined analogously to calculations done for the laboratory-scale experiments. Additionally, for Experiment 5 leachate samples were collected in addition to solid samples. This was used to calculate the difference between the pre- and post-digestion TS and VS masses. These masses were compared for the three LBRs where different inoculum approaches were evaluated (Fig. 11). VS reduction was within the range expected for this type of system and more successful than experiments 1-4 (Fig. 11). VS reduction values from the 10% top inoculation method (62% VS reduction) and the 10% mixed inoculation method (47% reduction) in this experiment compared favorably with VS reduction of the 10% inoculation method in the laboratory-scale experiment (50% reduction). The higher values noted in this experiment could be attributed to the readily degradable nature of the waste selected for this experimental batch (see Food Waste Collection section). The 0% inoculated batch in this experiment had a VS reduction of 49% while the laboratory-scale experiment 0% columns averaged 37% reduction. This result could also be due to the additional leachate inoculation in the demonstration-scale system, or possible unintentional inoculation from the use of the same equipment for handling the various batches which was difficult to clean at the demonstration scale.

Compared with the operating conditions of experiments 1-4, the operating conditions of experiment 5 was far more stable due to the replacement of faulty equipment within the system. Although there exist little substantial differences between the various batches of experiment 5, substantial VS reduction was observed for the 10% mixed inoculation, the 10% top inoculated and the 0% inoculum. pH ranges for experiment 5 system were also maintained in a narrow range between pH 7.5 and 7.75 without the use of chemical adjustment.
Figure 11. Results of the % volatile solids reduction from batch number 5. Error bars represent replicates of samples taken from the same batch.

The three inoculation approaches tested in experiment 5 appear remarkably similar (Fig. 11). However, in this experiment, there appears to be little difference between the mixed and the 0 % inoculum, while there seems to be a slight increase in the degradation in the top inoculated LBR. This is an unexpected result, as it is reasonable to assume that the hydrolyzing bacteria would inoculate the substrate more completely when the inoculum is fully mixed within the LBR. While results suggest top inoculation results in better performance compared to mixing and no inoculation, further testing is required to confirm this result. These results serve to support the findings from the laboratory-scale experiments, which indicate that leachate is acting as a significant transport mechanism for the hydrolyzing bacteria within the LBR. Further experimentation could seek to explore this finding in greater detail.

As leachate is the primary transport mechanism for organic compounds within the system, understanding the chemical quality of the leachate is an important way to interpret the extent of digestion process. Unlike the laboratory-scale experiments, in the demonstration-scale experiments (see Laboratory-scale Study), salts and ammonia were not artificially adjusted and remained low throughout the experiment. Conductivity values were typically below 1.0-1.1 mS/cm. This is in stark contrast to the higher values maintained in the laboratory-scale experiments (45 mS/cm). Ammonia values were similarly low. The values ranged between 120-250 mg TAN/L in the leachate samples. These values were far below the 3.5 g TAN/L maintained in the column experiments.

High chemical oxygen demand (COD) values were observed near the beginning of the test batch with these values tapering off as the substrate degraded (Fig. 12). This trend is
due to the action of the methanogenic bacteria within the system (predominantly the FF) which continually degrade many organic compounds within the leachate. The clear downward trend of the LST COD value indicated that although the single pass retention time of the FF was much lower than conventional systems, the constant recirculation of the leachate through the FF resulted in notable methanogenic activity.

It is important to note that not all hydrolysis byproducts are bioavailable to the methanogens. This resulted in the leachate accumulating organic molecules which were recalcitrant to further breakdown. This is observed after day 30 in the process when the leachate has reached a minimum concentration of organic compounds (Fig. 12). At that point, it appears that the system had reached an equilibrium where solid material in the LBRs did not substantially contribute to LST COD, and COD is the LST did not substantially decrease from methanogenesis in the FF. Also of note is the high COD within the leachate from the LST during the first ten days. This was likely due to leaching of the substrate in the preheating stage in the first six days of the process. Although it took nearly a week for the process to reach 32°C, there was likely psychrophilic degradation occurring during this period, as well as leaching of material solubilized from aerobic degradation prior to process start-up. Digestion at these low temperatures (< 30°C) is generally regarded for most substrates to be much slower than experienced in the 35°C range. In the case of this set of experimental batches, it is likely that the partially degraded potato waste, which made up a component of the food waste portion of the waste (see Materials and Methods), contributed to the initial increase in leachate COD.

**Figure 12.** Chemical oxygen demand (COD) of the leachate at the exit of the 3 LBRs and the LST tank. NI indicates not inoculated.
Biochemical methane potential (BMP) tests conducted in this experiment serve to provide information on the relative bioavailability of the various organic compounds in the leachate. The resulting values were used to generate a BMP:COD ratio ultimately representing the liters of methane produced per gram of COD. This is a useful measure to understand how biodegradable the COD is. An aqueous solution of glucose, which is a highly biodegradable organic compound, has a theoretical BMP:COD ratio of 0.35 L methane per g COD (Metcalf and Eddy, 2014). This value represents a typical value for bioavailable hydrocarbons. The BMP:COD ratios of the various leachate samples in this experiment (0.005 - 0.15 L methane per g COD) were substantially lower than the theoretical value (Fig. 13). Of note is that the BMP:COD ratio observed in samples collected from the LST may not be representative of the BMP:COD of leachate directly exiting the LBRs. The LST is a combination of effluent from LBRs and the FF, where organic matter in the LST is converted to methane (Fig. 2). Thus, some of the methane potential of liquid in the leachate is constantly removed via the recirculation between the LST and FF. Through each pass to the FF, readily biodegradable compounds are removed and non-degradable compounds accumulate. Early on in this experiment the leachate demonstrated a higher BMP:COD ratio than at the end, indicating the COD remaining at the end of the experiment was composed of a greater fraction of non-bioavailable organic compounds (Fig. 13). As these non-biodegradable compounds accumulate, the BMP:COD falls even further below the theoretical maximum value of 0.35 L methane per g COD. While BMP:COD measured in the LST was relatively low likely due to the recirculating nature of the system, the trend of BMP:COD indicates that organic matter in the LST was ultimately converted to methane (Fig. 13).

**Figure 13.** BMP:COD ratios for the various LBRs and the LST.
The BMP:COD ratios noted above (Fig. 13) were used to estimate the BMP values from the entire test, using the collected COD values (Fig. 12). This data was used to generate a relationship of COD to methane potential at various time points in an experimental batch (Fig. 14). In this interpolated data set, the LST BMP values start out higher than LBR effluent BMP, but then quickly drop much lower than the LBR values after day 10. This indicated that the FF was not fully degrading the LST COD until after 10 days into the experiment. Methanogenic bacteria (largely within the FF) served to reduce the COD of the leachate. During a period in the first few days of the process, the FF was unable to respond immediately to the influx of organic compounds within the leachate. This is to be expected in a process where all LBRs were started at the same point in time. A full-scale system will have LBRs that are operating at all stages of decomposition, and LBRs will be replaced gradually. Also of note is that the microorganisms in the FF were not acclimated to high loads of organic matter prior to experiment 5.

![Figure 14](image)

**Figure 14.** Estimated BMP of the leachate at each COD collection time point.

**Conclusions of demonstration-scale experiments**

In the demonstration-scale experiments, inoculation was not found to have a substantial impact on the solubilization of wastes within the LBR. This finding is likely due to the low salt and ammonia levels measured in the leachate compared to the laboratory-scale tests. Salts and ammonia were not adjusted as in the laboratory-scale experiments. Thus, the data indicate that inoculation is only critical when chemical conditions are not optimal (e.g., when inhibitors are present). Inhibitors may take months to build up in systems with leachate recycle, and thus, issues may not emerge early on. However, the laboratory-scale tests under elevated ammonia and salinity indicated a clear benefit. Thus, inhibitor levels should be monitored and if they are observed to be elevating, inoculation approaches can be used to maintain high performance. Further
experimentation, employing replicate reactors, increased experimental analysis, and longer-term (years) of operation could provide more complete data to help understand the details surrounding the disparity noticed between the laboratory- and demonstration-scale experiments.

There are important findings from the demonstration-scale experiments which complement previous results from the laboratory-scale experiments. Notably, the 0% inoculum control performed as well as the 10% mixed control, which indicates that leachate may serve as a transport mechanism for the hydrolysis inoculum in large scale systems. This finding has important implications for large-scale digestion projects. The imperative that initiated this study was to determine how to increase the rate of hydrolysis within the process in a cost-effective way. The use of large volumes of post-digestate within new batches is a technically viable method for inoculating new substrate, but this approach increases the total reactor volume for the system. Thus, the increased costs associated with this increased volume may end up negating any monetary benefits that inoculation may have. Combined solids- and leachate-based hydrolysis reactor inoculation using low volumes of solids could have the potential to cut reactor volume costs while still gaining some of the benefits that inoculation may have.

Taken collectively, data from the laboratory-scale and demonstration-scale studies lead to the following conclusions:

- Using digested solids as inoculum is beneficial when inhibitors are present and likely helps transfer critical microbes to the leachate.
- Leachate recirculation can effectively deliver critical hydrolyzing bacteria to LBRs.
- Combining solids- and leachate-based inoculation maximizes the number of bacteria contacting fresh waste and delivers a more diverse set of hydrolyzers to reactors.
- Enhanced leachate (i.e., introducing post-digestion solid inoculum into the leachate in an attempt to increase the concentration of critical hydrolyzers in the leachate) is a promising approach for increasing hydrolysis rates in high-solids AD processes, particularly under elevated ammonia and salinity conditions.

**Economic Analysis**

Economic analysis was conducted to address Objective 3. The purpose of this economic analysis was to evaluate profitability, or return on investment, of the multi-stage AD technology under development with respect to improved hydrolysis processes. This objective focused on conducting the economic analyses needed to guide industry decision making. The approach used was to evaluate economic indicators (operating profit, capital investment, and payback periods) with baseline assumptions for water and energy pricing and LBR solids digestion times at three potential system scales. Then, the impact of varying digestion time, water prices and energy prices was investigated to
determine sensitivity to these parameters. Improving hydrolysis rates via advanced microbial management strategies would have the effect of reducing required solid residence times, which would result in potential for increasing unit loading rate or a reduction in size of equipment. Thus, the lower residence times evaluated are indicators of the impact of the technology advances developed herein on process economics. For comparison purposes we standardized all systems based on MMBtu biogas produced.

The conceptual design for the multi-stage AD technology developed at CSU was scaled to that of existing full-scale AD systems to investigate the impact of technology advances on economic feasibility. The conceptual design for the CSU AD technology is based on a modular design in which leachate modules (LMs) served as the LBR. The LMs were assumed to be 5700-gal and would be loaded at various waste generation sites and transported to a central location to be integrated into the multi-stage AD system. Existing system 1 uses a single-stage dry digestion technology to produce biogas from food waste and yard waste and generates 73,036 MMBtu/yr. System 2 is a multi-stage wet digester that produces biogas from food waste and manure that generates 985,500 MMBtu/yr. System 3 is the planned scaled up, next phase of System 2 and produces 2,555,000 MMBtu/yr. The economic analysis included estimation of the one-time capital investment, revenues from biogas sales, and selected annual operating costs. The production costs and revenues from generation of co-products such as compost were not included. This analysis does not include fixed costs as well as a detailed operating cost section due to the lack of data availability. Fixed costs include depreciation, mortgage payments for land, interest expense, and insurance expense. Excluded operating costs included any licenses or permits needed for operation, feedstock procurement, and inoculum procurement.

The capital investment was divided into six categories which included tanks, instrumentation, LBRs, pumps/HVAC system, plumbing, and the generator set. The costs associated with each system are presented in Table 6. Scale 1 requires 10 LBRs, scale 2 requires 105 LBRs, and scale 3 requires 270 LBRs due to the modular design of the CSU developed technology. Actual reported overall capital costs were ~$5 million, $80 million, and $100 million for full-scale systems 1, 2 and 3. Thus, calculated capital costs for the multi-stage dry digestion technology studied here compare favorably with reported capital costs for 2 of the full-scale systems used as a basis of comparison (scale 1 and 2). Capital costs reported in Table 6 were estimated and actual costs may differ from these. Operating costs were not available for full-scale systems, and thus it was only possible to make a simple comparison of capital costs.
Table 6. Capital investment costs for 21 day digestion across scales

<table>
<thead>
<tr>
<th>Capital Investment Categories</th>
<th>Scale 1</th>
<th>Scale 2</th>
<th>Scale 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water tap fee</td>
<td>50,000</td>
<td>50,000</td>
<td>50,000</td>
</tr>
<tr>
<td>Tanks</td>
<td>1,293,891</td>
<td>17,458,914</td>
<td>45,263,852</td>
</tr>
<tr>
<td>Instrumentation</td>
<td>539,168</td>
<td>7,115,070</td>
<td>18,425,662</td>
</tr>
<tr>
<td>LBRs</td>
<td>330,000</td>
<td>4,456,000</td>
<td>11,550,000</td>
</tr>
<tr>
<td>Pumps/HVAC</td>
<td>1,120,979</td>
<td>15,084,746</td>
<td>39,103,222</td>
</tr>
<tr>
<td>Plumbing</td>
<td>260,428</td>
<td>3,268,970</td>
<td>8,441,678</td>
</tr>
<tr>
<td>Generator Set</td>
<td>250,000</td>
<td>1,815,000</td>
<td>4,950,000</td>
</tr>
<tr>
<td><strong>Total Investment</strong></td>
<td>3,844,465</td>
<td>49,284,699</td>
<td>127,784,415</td>
</tr>
</tbody>
</table>

| Total Investment Per MMBtu sold in 1 year** | 58.49 | 55.53 | 55.57 |

*MMBTU generated: Scale 1 = 73,036 MMBtu/yr, scale 2 = 985,500 MMBtu/yr, scale 3 = 2,555,000 MMBtu/yr
**MMBTU sold: Scale 1 = 65,732 MMBtu/yr, scale 2 = 886,950 MMBtu/yr, scale 3 = 2,299,500 MMBtu/yr

In addition to the one-time sunk cost, a number of operating costs are needed to generate the energy. Operating costs were split into 4 categories: water, utilities, labor, and maintenance. All economic assumptions are presented in Table 7 with a detailed description following. Water costs were estimated based on a daily water use with a one-time $50,000 fee for initiating the tap. Water use was estimated at 11.0 ft$^3$/day, 149 ft$^3$/day, and 386 ft$^3$/day for scales 1, 2, and 3; respectively. Commercial water rates were collected from national averages with a base price of $1,355.58 per month with a $2.26/1000 gal usage charge.

Utility costs were estimated using a 10% parasitic load. Since the energy needed to run the system was generated by the system, utility costs were valued at $0. The remaining 90% of energy generated was assumed to be sold at $6.09/1000 ft$^3$, which is half the price of the average 2014 commercial rate paid for natural gas (eia.gov). Valuing the energy selling price at half the commercial rate is a common practice since utility companies rarely pay the current market price to purchase alternative energy sources. The number of full-time employees varied based on the size of the AD system. A $20/hr labor rate was used for each system. Maintenance costs vary substantially based on systems and management preferences. This makes it difficult to collect actual costs. We assumed the annual maintenance cost was equivalent to 1% of capital investment costs to provide a conservative estimate.
Table 7. Baseline economic assumptions for annual operating costs with a 21 day digestion time

<table>
<thead>
<tr>
<th>Assumptions</th>
<th>Scale 1</th>
<th>Scale 2</th>
<th>Scale 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water use (1000 gal/year)</td>
<td>745</td>
<td>10,054</td>
<td>26,065</td>
</tr>
<tr>
<td>Water price-monthly base charge ($)</td>
<td>1,356</td>
<td>1,356</td>
<td>1,356</td>
</tr>
<tr>
<td>Water price ($/1000 gal)</td>
<td>2.26</td>
<td>2.26</td>
<td>2.26</td>
</tr>
<tr>
<td>Natural gas selling price ($/1000 standard ft³)</td>
<td>6.08</td>
<td>6.08</td>
<td>6.08</td>
</tr>
<tr>
<td>Full time employees (number)</td>
<td>3</td>
<td>14</td>
<td>22</td>
</tr>
<tr>
<td>Full-time employee labor rate ($/hr)</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Maintenance cost (% investment cost)</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
</tr>
</tbody>
</table>

A simplistic base-line operating profit estimate was calculated as a function of revenue and operating costs. The operating profit shows the firm’s ability to meet short term obligations. This estimate does not include fixed costs (e.g. depreciation, interest expense, mortgage payments, and insurance) due to the limitations of available data. However, including a fixed cost estimate provides a complete profit estimate and allows for long-term planning decisions. The final profit numbers will change as a function of the fixed costs for the system.

Results are presented in Table 8. Revenue was estimated assuming a biogas selling price equal to $6.08/1000 ft³. Typically, the sale of energy is at a discounted rate compared to what the commercial or residential consumer pays. This price can range from 30-50% of the commercial and residential price; for the purposes of this analysis we assumed it was 50% lower. The base-line profit was estimated by subtracting the operating costs from the revenue. Operating costs varied by the scale of the system.

The operating profit is approximately 54%, 79%, and 83% of the revenue generated for scales 1, 2, and 3, respectively.

Table 8. Base-line operating profit estimates for 21 day digestion

<table>
<thead>
<tr>
<th>Capital Investment Categories</th>
<th>Scale 1</th>
<th>Scale 2</th>
<th>Scale 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Revenue $/year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Power Generation</td>
<td>400,000</td>
<td>5,402,000</td>
<td>14,004,000</td>
</tr>
<tr>
<td>Operating Costs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>18,000</td>
<td>39,000</td>
<td>75,000</td>
</tr>
<tr>
<td>Labor</td>
<td>125,000</td>
<td>582,000</td>
<td>915,000</td>
</tr>
<tr>
<td>Maintenance</td>
<td>38,000</td>
<td>492,000</td>
<td>1,278,000</td>
</tr>
<tr>
<td>Total Operating Costs</td>
<td>181,000</td>
<td>1,114,000</td>
<td>2,268,000</td>
</tr>
<tr>
<td>Operating Profit ($/MMBtu Sold)</td>
<td>219,000</td>
<td>4,288,000</td>
<td>11,736,000</td>
</tr>
<tr>
<td>Payback Period (years)</td>
<td>17.55</td>
<td>11.49</td>
<td>10.89</td>
</tr>
</tbody>
</table>
The digestion solid waste residence time affects the overall investment and operating costs of this system. To capture the impact of residence time on overall economics, 5 LBR residence times were studied: 14, 18, 21, 25, and 28 days (Fig. 15 and 16). Fig. 15 presents the capital investment cost across the 3 scales as a function of the digestion time periods. Due to different sizes across the 3 scales, total capital investment costs were standardized by the MMBtu sold in 1 year for the purposes of comparison. Relative capital investment costs are consistently higher for scale 1 compared to scale 2 and 3. Capital costs per MMBtu sold in one year for scale 2 and 3 are relatively close to each other across all digestion time periods due to similar scaling between their power generation and operating costs (i.e., genset costs are relatively constant per kw between these two scales).

![Figure 15. Capital investment costs per MMBtu sold in one year.](image)

The annual operating profits for the baseline economic assumptions are presented in Fig. 16 for the various days in digestion. Annual operating profit remains relatively stable over the various LBR retention times for each scale with Scale 3 having the largest operating profit potential.
The payback period for an investment determines how many years it will take to recover the initial investment after paying all expenses. This is a simplistic approach which does not consider the time value of money nor the timing of when cash flows are received. However, the payback period allows investors to estimate how long it will take to earn back the initial investment assuming the assumptions defined hold constant. Using the baseline economic assumptions reported in Table 6 and Table 7, the average payback period varies from 10-19 years depending on the scale and LBR solid residence time (Fig. 17). All scales have their quickest payback period with a 14-day digestion residence time, as expected. Further, at all scales the payback period was decreased by 12% by reducing the solids residence times according to the analysis performed herein. Although the reduction in payback period due to improved hydrolysis performance and reduced required residence time is minimal, results indicate that hydrolysis process improvements do have an impact on overall economics. Further technology advancements are desired to further reduce capital costs and payback periods.
An analysis was completed to determine the extent that capital investment costs would need to decrease to achieve a Payback Period equal to 5 years with a $6.08/MMBtu biogas selling price. Results demonstrate that capital investment costs would need to decrease by a minimum of 51%; however for Scale 1, costs must decrease by 70-73% (Fig. 18). Due to the relationship between paying all expenses and recovering the initial investment, each scale could improve this capital investment decrease by evaluating their operating costs. Specifically, if operating costs are lower, they have more profit available to recover the initial investment at a quicker rate.

Figure 17. Payback period of capital investment using baseline economic assumptions

Figure 18. Capital investment cost decrease for Payback Period of 5 years using baseline economic assumptions
Fig. 19 shows the results of a changing biogas selling price on the payback period while holding all other baseline economic assumptions constant. Four biogas selling prices were chosen: $3.04, $6.08, $12.16, and $15.30/MMBtu. Currently, on average consumers are paying $12.16/MMBtu, nationally. Therefore, the most realistic selling price for an energy producer is 50% of the consumer price, which is $6.08/MMBtu. However, gas prices vary significantly across the country and change over time. The $3.04/MMBtu causes Scale 1 to have a very large payback period of 206 years; however, the payback period decreases substantially as the selling price of biogas increases. At $15.30/MMBtu the payback period varies from 5 years for Scale 1 to 4 years for Scale 3.

![Figure 19. Payback period for 4 selling prices, holding all other baseline economic assumptions constant.](image)

Water prices can fluctuate greatly across the U.S. Fig. 20 presents the payback period for various water prices holding all other baseline economic assumptions constant. The fixed base monthly price for water did not change, rather the variable $/1000 gallon water price increased. The baseline water price of $2.26/1000 gal was increased by factors of 2, 3, 4, and 5 to determine its effects on the payback period and investigate the impact of water price variation. Results show that payback periods increase with increased water prices; however, the changes are negligible within the systems. This demonstrates that water prices are not the determining factor when evaluating the economic viability of these systems.
Conclusions of economic analysis

This economic analysis concludes that the largest obstacle to adopting this digestion system is the large initial capital investment costs. The payback periods for this investment are greater than 10 years across all scenarios at baseline energy costs due to low operating profit estimates compared to the capital investment. For higher energy costs, payback periods of 4-6 yrs were estimated. It is also important to emphasize this analysis does not account for all operating and fixed costs due to data limitations. Estimated payback periods will only increase in value as these additional expenses are accounted for in future analysis. However, overall capital costs for the system investigated were in the same range as those reported for existing full-scale systems. Further, analysis suggests that improving hydrolysis rates and therefore reducing LBR residence times results in a reduction of capital costs. Therefore, the economic analysis indicates that advancing hydrolysis technologies will improve overall AD economics. However, further advancements are needed to improve economic viability when energy prices are low.
Conclusions

Advances in technologies for hydrolysis of OFMSW are needed to improve economic viability. This study investigated the use of advanced microbial management strategies for improving hydrolysis within multi-stage AD systems. In particular, the use of acclimated inhibitor-tolerant inocula was studied. To inoculate each waste batch, digested solids from the previous batch were used and performance was compared for various amounts of inoculum. Research showed that high quantities of inoculum (~60%) increase waste hydrolysis and are beneficial when inhibitors are present (>1.7 g TAN/L and 3.5 g Na+/L). After start-up (~112 days) with high inoculum quantities, leachate recirculation leads to accumulation of specific inhibitor-tolerant hydrolyzing bacteria in leachate. During long-term operation, low inoculum quantities (~10%) effectively increase waste hydrolysis relative to without solids-derived inoculum. Combining leachate recirculation with inoculation with low quantities of digested solids was found to lead to the best performance at laboratory-scale. Adding a low quantity of digested solids (10% by mass) was found to improve performance because it doubled the quantity of bacteria contacting waste over a batch. Additionally, the digested solids inoculum provided different microbes than recirculated leachate; in particular digestate provided Clostridia, which are known to hydrolyze cellulose. Demonstration-scale experiments did not show the same benefits of digestate inoculation as at laboratory-scale. However, this was likely due to lower salinity and ammonia levels in the demonstration-scale system. At the demonstration-scale, reactor start-up was conducted similarly to how it would be done at full-scale, and over the operational periods (6 months), inhibitors had not yet built up. Thus, research suggests that the benefits of inoculation may only be significant when high levels of inhibitors are present. However, additional large-scale studies would be needed before firm conclusions can be drawn. Such studies could be run for a longer term (multiple years) or leachate inhibitor concentrations could be artificially altered. Economic analysis showed that improvements in hydrolysis rates will decrease capital cost; at all scales the payback period was decreased by 12% by reducing the solids residence times. Further advancements are still needed to improve profitability when energy prices are low.
Materials and Methods

Laboratory-Scale Study Methods

Feedstock and inoculum collection

The OFMSW feedstock utilized in this study was a combination of food and yard waste. Food waste was collected from the CSU Ram’s Horn dining facility as previously described (Wilson et al., 2013). Food waste was stored in bins for less than 2 days prior to experimental use. Yard waste (leaves and grass clippings) and wood chips (used as a bulking material) were collected from a local landscaping company (Hageman Earth Cycle, Fort Collins, CO). After collection, OFMSW was stored at 4°C until subsequent use the following day. Immediately prior to experimental use, food waste (82% by wet mass), grass clippings (7.25%), leaves (3.5%), and wood chips (7.25%) were mixed thoroughly by hand to create the OFMSW feedstock loaded into LBRs. This food waste to yard waste ratio is consistent with ratios that may be used in the field (Beanie, 2013). Original microbial inoculum was collected from the Drake wastewater treatment plant (DWWTP) mesophilic anaerobic digester (Fort Collins, CO) and used immediately after collection. Waste and inoculum were analyzed for TS and VS content prior to digestion (Table 9).

<table>
<thead>
<tr>
<th>Table 9. Characteristics of waste and inocula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>Food waste</td>
</tr>
<tr>
<td>Grass clippings</td>
</tr>
<tr>
<td>Leaves</td>
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<tr>
<td>DWWTP Inoculum</td>
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</tbody>
</table>

*Numbers in parentheses indicate standard deviations of 2-3 replicate batches (waste) or samples (inoculum).

Reactor configurations

For experiments focused on reactor start-up, 4 LBRs were connected to individual leachate storage tanks (LSTs) without connections to methanogenic reactors (Fig. 3 a, d). LBRs were clear polycarbonate pipe with a diameter and height of 20 cm and 91 cm, respectively. The LBRs were sealed with Cherne adjustable pipe caps that were fitted with 5 evenly-spaced ports for leachate distribution (Fig. 3b). The volume of waste in each LBR varied depending on the percentage of inoculum used and was ~10 - 25L. 5-gal plastic carboys (~4 gal leachate) were utilized as LSTs. Leachate from each LBR was
gravity-fed into an individual LST, and then pumped (via Cole Parmer Masterflex L/S Digital Drive peristaltic pumps) back to the top of the LBR at a rate of 20 ml/min. Flow rates were digitally controlled, and the accuracy of the flow rate was manually confirmed three times during the experimental period. 1.27 cm and 0.32 cm vinyl tubing were used for leachate delivery to the LSTs and LBRs, respectively.

For long-term operation, a multi-stage system was utilized that contained a triplicate set of LBRs (described above), 1 LST (54.6 cm x 38.1 cm x 66 cm), and 1 FF reactor (cylindrical drum with a radius and height of 23 cm and 79 cm, respectively) (Fig. 3c). The LST and FF had working volumes of ~10 and 30 gal, respectively. Leachate from the 3 LBRs flowed into 1 LST and then was pumped to the FF at a rate of 9 ml/min. Effluent from the FF was pumped at the same rate back to the LST. Leachate also was pumped from the LST to the top of the LBRs as described above. Vinyl tubing (1.27 cm inside diameter) was used for leachate delivery to and from the FF. The FF was filled with plastic packing material (Bioportz moving media, Entex Technologies). Leachate samples for liquid analysis were collected from the LST and FF reactor effluent (post-treatment). Samples were collected from the LST, rather than LBR effluent directly, as a means of monitoring pH and VFA concentrations in the composited leachate delivered to the LBRs and FF to ensure conditions were not inhibitory (e.g., >6.5 pH in the LST) (Ahn et al., 2010).

**Microbial acclimation for LBRs and FF**

To develop a large volume of acclimated inoculum to seed LBRs for tests under elevated ammonia and salinity concentrations, 2 LBRs were loaded with 3600 g (wet weight) of OFMSW and inoculated with DWWTP AD sludge (20% of total material by mass) by layering. During digestion, leachate generated was collected in one LST (initially filled with nitrogen-purged deionized [DI] water) and recirculated back to the top of each LBR using the configuration in Fig. 3a. Leachate was analyzed at least twice a week for DCOD, VFAs, ammonia, pH, and EC. After the first batch (16 days), the LBRs were emptied and refilled by layering fresh waste with previously digested waste (60% wet weight). Fresh waste and inoculum were alternately layered (4 and 5 layers of fresh waste and inoculum, respectively) to provide contact between waste and inoculum; layers were separated by mesh (5 mm) so that they could be distinguished post-digestion. Fresh waste layers were 900 g (wet weight) each, and the mass of inoculum layers varied depending on the inoculum percentage utilized. Columns were purged with nitrogen gas after each refilling to avoid prolonged exposure of the inoculum to oxygen. LBRs were operated over a 16-week period, and ammonia and salinity concentrations in leachate gradually were elevated artificially to 3.5 g TAN/L and a conductivity of 45 mS/cm (surrogate measure of salinity, ~6 g Na+/L), respectively, by adding ammonium chloride and sodium chloride. Acclimation was considered complete when final solids destruction did not vary by more than 10% for consecutive batches.
To acclimate a microbial inoculum for the FF reactor (Fig. 3c), AD sludge was incubated at 35 ºC for 2 weeks and fed glucose while the ammonia and salinity concentrations were increased gradually to 1.5 g TAN/L and 3 g Na+/L using ammonia chloride and sodium chloride. Next, the inoculum was mixed with a nutrient solution (50% v/v basis) (Owen et al., 1979) to ensure that nutrients were not limiting in the system, and the reactor was fed glucose or leachate generated from the LBRs at an organic loading rate (OLR) of 1 g COD/L-day while ammonia and conductivity concentrations were slowly increased further to 3.5 g TAN/L and 45 mS/cm. The effluent was recycled continuously at a rate of 50 ml/min, and the pH was adjusted to 7 using NaOH as needed. Throughout the 16-week period, the methanogenic reactor was operated as a “wet” (low-solids) digester (i.e., recycled liquid flowed through a sludge of suspended biomass) rather than as a FF reactor to avoid sub-optimal microorganisms (non-inhibitor-tolerant microorganisms) attaching to the plastic packing. After 16 weeks, packing material was added to the reactor, and the FF reactor was operated for 3 weeks prior to subsequent experiments.

**Reactor operation**

To determine optimal seeding methods at start-up or when inhibitors become elevated, LBRs operating under elevated ammonia and salinity (3.5 g TAN/L and 45 mS/cm, respectively) were seeded by layering initially with unacclimated (60% by wet mass) or different percentages of acclimated inoculum (60%, 10%, and 0% by mass) and operated for a total of 3 batches with the set-up described above (Fig. 3a). The unacclimated inoculum utilized was previously digested waste from an identical multi-stage system operating under low ammonia and salinity. The source of acclimated inoculum was previously digested waste from the acclimated LBRs. For all 3 batches, the mass of fresh waste (3600 g) loaded into each LBR remained constant. Since a FF reactor was not utilized in this set-up, leachate was manually diluted 50% by volume daily (Xu et al., 2011) with nitrogen-purged DI water to simulate the FF function (i.e., to reduce the concentration of soluble organics typically consumed in the FF). The DI water was spiked with appropriate concentrations of ammonium chloride and sodium chloride to maintain elevated ammonia and sodium in the system.

To determine the optimal ratio of previously digested waste to fresh waste over time, LBRs operating under elevated ammonia and salinity were seeded initially with a high percentage (60% by mass) of acclimated inoculum with the set-up described above (Fig. 3c). After each batch (16 days), the LBRs were emptied and refilled using previously digested waste. Then, LBRs were reseeded with decreasing percentages of inoculum (40%, 20%, 10%, and 0%) to determine the minimum amount of inoculum needed to maintain optimal microorganisms in the system. For each inoculum percentage, LBRs were operated for 2 - 3 batches. Post-digestion, separate inoculum and digested waste layers were mixed thoroughly by hand for 5 minutes, and 5 random samples were analyzed for TS and VS. Thus, for each inoculum percentage, hydrolysis performance results were based on a minimum of 30 samples (3 columns x 2 batches x 5 samples).
Chemical analyses

Initial (pre-digestion) and final (post-digestion) TS and VS percentages of the substrate were measured and calculated according to EPA Method 1684. These percentages were multiplied by the total mass of the substrate in the reactors at the beginning and end of the experiments to yield the total mass of TS and VS pre- and post-digestion, respectively. To determine the removal efficiency for VS (% VS\text{reduction}), the following equation was used:

\[
%\text{VS}_{\text{reduction}} = \frac{m_{\text{vs(initial)}} - m_{\text{vs(final)}}}{m_{\text{vs(initial)}}}
\]

where \(m_{\text{vs(initial)}}\) and \(m_{\text{vs(final)}}\) are the total masses of VS in each reactor pre- and post-digestion, respectively. Liquid samples extracted from the LST and FF effluent were analyzed for pH and EC using probes (VWR #89231-604 and #11388-382, respectively). DCOD concentrations were determined using Hach’s COD High Range Vials and digestion colorimetric method (Hach, Loveland, CO). VFAs were determined using Hach’s Volatile Acids TNTplus Reagent Set and esterification method. Samples were filtered through a 0.2 μm syringe filter prior to DCOD and VFA analyses. Hach’s Nitrogen-Ammonia High Range Reagent Set was utilized weekly to verify that ammonia concentrations remained constant over time. Biogas volume was measured utilizing a wet tip gas meter (Speece Co., Nashville, TN), and biogas composition was determined via gas chromatography using a thermal conductivity detector as described previously (Wilson et al., 2013).

Microbial analyses

Motivated by the long-term study results, experiments were conducted to compare quantities and compositions of the microbial communities among the leachate, inoculum, and fresh waste. Samples used for quantification of Bacteria in leachate and previously digested inoculum analyses were collected on day 192 of the long-term experiment. To prepare samples for DNA extraction, 50-ml leachate samples were centrifuged at 5,000 g for 10 minutes at 4°C. The supernatant was discarded, and the remaining pellet was used for DNA extraction. For solid samples, 25 g of fresh waste or inoculum were blended with 125 ml of sterile DI water for 1 minute to ensure representative samples. The blended mixture was then centrifuged at 5000 g for 5 minutes at 4°C. The supernatant was discarded, and the pellet was used for DNA extraction. For all samples, DNA was extracted using MoBio’s PowerSoil® DNA isolation kit according to the manufacturer’s instructions.

SYBR green™ assays were utilized to quantify the number of bacterial 16S rRNA gene copies in each sample. Genomic DNA extracted from \textit{Thauera aromatica} (ATCC #7002265D) was used to generate calibration curves. The primer set 1369F and 1492R was used for amplification (Li et al., 2010), and all assays were conducted using an ABI
7300 real-time PCR system (Applied Biosystems, Foster City, CA). Each 25-µl SYBR green™ qPCR reaction contained: 1X Power SYBR green™ (Life technologies, Grand Island, NY), forward and reverse primers (0.15 µM), PCR grade water, and 4 ng DNA template. Thermocycling conditions were as follows: 95ºC for 10 min, followed by 40 cycles of 95ºC for 15 s, 56ºC for 20 s, and 68ºC for 30 s (Li et al., 2010). Dissociation curve analysis was conducted to confirm amplicon specificity. For all inoculum percentages, bacterial 16S rRNA gene quantities were estimated based on a single sample (192-day sample) to eliminate the impact of changes over time. The number of 16S rRNA genes per mass of solid inoculum was determined, and the total quantity of Bacteria added to the LBRs was projected for each inoculum percentage. All numbers are expressed as a function of the amount of fresh waste added, which was constant over time. For leachate, the quantity of 16S rRNA gene copies was determined per volume of leachate, and multiplied by the amount of leachate distributed to the LBRs over the experimental period.

Community composition was determined via T-RFLP analyses of DNA isolated from leachate, fresh waste, and previously digested inoculum and waste. Previously digested waste was collected from three LBRs operated with fresh waste and inoculum (40% by mass) layered (LI), fresh waste and inoculum (40% by mass) thoroughly mixed by hand (MI), and fresh waste only with no inoculum (NI) utilizing the set-up described above for long-term operation studies. To determine microbes present early in the batch period, digested samples were extracted from each layer of the LBRs after 5 days of operation; leachate and previously digested inoculum samples were collected as described in this section. To track changes to bacterial, fermenting, and hydrolyzing communities, T-RFLP analyses were conducted targeting the 16S rRNA gene, hydA gene (encodes the alpha subunit of iron hydrogenase), and cel48 gene (encodes glycoside hydrolases of family 48). T-RFLP analysis targeting the 16S rRNA gene was conducted as described by Wilson et al. (2013). T-RFLP analyses targeting the hydA gene and cel48 gene were conducted as previously described by Lefèvre et al. (2013).

DNA from leachate (collected on day 192), fresh waste, and inoculum (collected on day 192) were submitted to Research and Testing Laboratories LLC (Lubbock, Texas) for bacterial 16S rRNA gene amplicon sequencing using Illumina MiSeq. Primers 28f and 388R were used, targeting the V1-V2 hypervariable regions of 16S rRNA genes (Sundquist et al., 2007; Turnbaugh et al., 2009). Bioinformatic analysis was conducted as reported previously (Wilson et al., 2016).

**Statistical analyses**

ANOVA and Tukey’s HSD tests were conducted using R Statistical Software (R Core Team, 2012) to determine if the slope of the fitted regression line for VS reduction as a function of inoculum percentage was statistically different from 0 as well as to determine statistical differences in average VS reduction for a range of inoculum percentages.
Results yielding a p-value less than 0.05 were considered to be significant. T-RFLP data were analyzed and similarity matrices were calculated according to Wilson et al. (2013), except terminal restriction fragments (T-RFs) representing less than 1% of the total area were excluded (Rees et al., 2004). Pairwise distances were calculated aligned sequences, followed by clustering and estimation of Shannon diversity.

Demonstration-scale Methods

Demonstration-scale multi-staged anaerobic digestion system

The MSAD constructed at CSU was designed as a mobile demonstration of the technology. The mobile system can be dispatched to different locations, as it is mounted onto a 8’ wide and 48’ long refrigerated transport trailer, (Fig. 21).

The facility is equipped with:
- Six mobile LBRs 60-gallon capacity in each LBR
- Three LBR docking stations, each capable of delivering and draining two gpm from the LBRs
- Loading dock and hoists for maneuvering the LBRs
- One 300 gallon LST
- One 300 gallon FF with 30 ft³ of BioPortz media (Entex Technologies)
- Two 275-gallon gas storage tanks
- Gas flare and generator to dispose of the gas produced
- Dedicated control room to house the system electrical panels and controls
- A programmable logic controller (PLC) to monitor and control the process
- Separate control room and column lab to facilitate column experiments

Figure 21. View of the Front of the CSU Demonstration Scale MSAD
The control room is located at the front of the trailer (Fig. 22, far left). The control room houses many important functional components of the system, including heating and ventilation, electrical panels, and the system’s PLC. The reactor room is the area where the LST tanks, FF tanks, and the gas storage tanks are housed (Fig. 22, right of control room). Beside these tanks are the LBR docking stations (Fig. 22). Lastly, a column scale laboratory is in the back of the trailer (Fig. 22). The columns scale laboratory did not support experiments for this project.

![Figure 22. Depiction of the CSU Demonstration Scale MSAD](image)

This system is configured to operate with six, 60-gallon portable LBR modules. However, within the demonstration scale system, only needs 3 LBR docking stations are for use during operations. These stations are used so that three LBRs can be docked while the other three are being cleaned and pressure tested before the next experimental batch.

The demonstration-scale system uses three different pumping stations to circulate leachate in the system: one for pumping leachate from the LST to the LBRs, one for pumping leachate from the LST to the FF, and one for returning the leachate from the sump pit back to the LST (Fig. 23). Leachate is drawn from the LST (300-gallon tank) by a series of pumps located on the top of each sealed LBR (Fig. 23). In this configuration, two gpm of leachate can be pumped from the LST to the top of the LBR where it trickles down through the substrate inside. The resulting liquid that drains from the LBRs is collected into a sump pit (Fig. 23). Once the sump pit fills sufficiently with leachate, a 2,200 gph submersible sump pump returns the leachate to the LST (Fig. 23).
The LST also serves as a source of leachate for the FF (Fig. 23). The FF (300-gallon tank) is equipped with a dedicated leachate supply pump (2 gpm) that pumps leachate from the LST to the top of the FF. Liquid from the LST is recycled through the FF near 4.5 times per day, for a typical retention time near 9 hours. The FF and LST are hydraulically connected near their bases. This allows liquid to return under gravity to the LST, thus completing the recycle loop. Within the system, this recycle process is repeated continuously (approximately 45 times) over the course of a 21-day digestion cycle. Salinity and ammonia levels were allowed to build up naturally, with only occasional dilution water added to refill the Leachate Storage Tank as needed.

**Detailed demonstration-scale system description**

There are several subsystems operating in tandem in the demonstration-scale system. The following components will be covered in detail: control systems, LBR leachate system, FF leachate system, gas handling system, process heating system, environmental and safety systems, and process support systems.

**Control System**

The demonstration-scale system is equipped with a control system capable of monitoring and controlling many points within the trailer. The control system is composed of four main parts: field devices, the in/out (I/O) modules, the PLC, and the human machine interface (HMI). Together, these components operate to enable process automation as well as remote monitoring and control. The PLC, HMI, and I/O modules used for this
project were all manufactured by EZ Automation while the field devices (e.g. sensors, switches, and control devices) were selected from a multitude of manufacturers. Process data is collected through the use of various field devices, namely sensors and switches, and there is great diversity in the devices. The control system uses these devices to either gather process data (input devices) or to support environmental and safety related systems (output devices). Examples of devices for process data monitoring include temperature sensors, liquid level switches, and pH sensors. These devices are configured to be monitored on a continuous basis. However, the primary impetus for using a control system is to control field devices, not just to monitor them. As such, the input signals are received by the control system and the programmed response is carried out by sending control signals to output devices. Common output devices in this process are pumps, lights, fans, and alarms.

The various field devices are located in various locations throughout the process, but they are all wired into the I/O modules. The I/O modules are removable devices that plug into the PLC during use. I/O modules act as an intermediary between the PLC and field devices. The I/O modules electrically isolate the PLC and field devices from one another, while still relaying signals between them (Fig. 24).

![Figure 24. Eight modules plugged into a PLC base](image)

The PLC is a configurable control system which enables logic based control of the process. Custom control programs specifically generated for the demonstration-scale unit were uploaded to the PLC system. The PLC uses this program to interpret input signals from field devices as well as provide output signals to control devices. In this installation, two separate PLCs are provided. These PLCs are connected and operate as a single control unit through the use of a protocol known as Modbus. The Modbus protocol requires the designation of master and slave units, which enables the devices to share information and commands between the units.

The HMI enables operators to interface with the process both locally and remotely. Locally, operators may utilize the touch panel HMI (EZ Automation EZ Dura-panel 6”) to
access a custom graphical interface (Fig. 25). The graphical user interface developed for this process is composed of over 30 separate slides which display various variables from the process. Using this local HMI, the status of the system can be monitored and controlled based on operator input. This same graphical interface may also be controlled remotely from a computer located on the same network. Remote monitoring and control software, provided by the manufacturer, enables access of the HMI from a computer connected to the network via Ethernet. This access point serves the same basic functions of the local interface. Utilizing commercially available remote desktop software, this computer and subsequently the HMI, may be accessed from off-site locations. With this functionality, the demonstration-scale system may be accessed for monitoring and control purposes from anywhere a suitable web connection is available.

Figure 25. Human machine interface- EZ automation EZ Dura-panel 6.”

The Leach Bed Reactor System
The three LBRs within the CSU demonstration-scale MSAD serve as the primary location for substrate hydrolysis. Process stability is contingent on transporting the soluble hydrolysis products need to be transported out of the LBR. Facilitated through cycling leachate through the LST and the LBRs. Leachate derived from the LST is filtered, chemically treated with caustic to raise the leachate pH if needed, and pumped into the LBR. The leachate picks up organic products here and flows under gravity to a collection sump where it is pumped to the LST once again. The detailed process is outlined below.

LBR Leachate Pretreatment and Distribution
Under normal operations, stored leachate passes out of the LST through a ball valve located on the side of the tank. The water passes through a 0.75” flexible vinyl hose into the pretreatment area of the front control room (Fig. 26).
Leachate chemical and physical pretreatment equipment is installed in this system. Chemical pretreatment is used to allow monitoring and control of leachate pH. A pH transmitter is equipped to monitor and report pH values to the PLC. The PLC was programmed to initiate dosing of an aqueous 1 M NaOH solution when pH values fell below 7.25. pH adjustment was in practice only used during the first 30 days of system startup while the FF was acclimating to the leachate produced by the LBRs. Physical pretreatment is provided through a two-step filtration system to remove particulates in order to protect downstream components from unnecessary wear and failure.

Leachate then passes through a canister filter (Pur Flo 10-micron pleated filter; Fig. 27). This canister filter is connected with isolation valves and union disconnects on both sides. The lid of the canister is equipped with a gas removal port. This is a custom modification to allow removal of the gasses that tend to come out of solution inside the filter. As the liquid passes out of the canister filter, it enters an inline pH sensor where the pH is measured. In this configuration, the effects of the pH adjustment dosage are delayed considerably from the actual time of dose at this post filter location due to the dissolution and buffering within the filter.
After the pH detector, leachate passes into the LBR pump leachate manifold (Fig. 28). Here, leachate is distributed to three identical branches that distribute liquid to each LBR. Each branch has a PVC gate valve (Fig. 29), a stainless steel inline pre-filter (1/2" NPT 80 mesh; Fig. 30), and connection to leachate feed pumps (detailed in the following section).
Leachate Pumps
Following pre-filtration, the leachate enters the LBR leachate delivery pump. The selected pumps are low-voltage DC pressure-demand equipped diaphragm pumps (NorthStar model # 2682271) which deliver 2 gallons per minute at up to 70 psi (Fig. 31). Diaphragm pumps are commonly used for chemical dosing and high-pressure water delivery applications, but they are usually poorly suited for conventional digestion effluents due to clogging from suspended particulates. However, the leachate drawn from the LST is very low in suspended solids and requires only minor pre-filtration to bring the water to a quality suitable to be reliably pumped with the diaphragm pumps (LBR Leachate Pretreatment and Distribution).

The diaphragm pumps are oversized for this particular application, but smaller diaphragm pumps were discontinued from use after reliability issues were encountered related to small particles. Without motor speed control, the flow rate of 2 gpm equates to a leachate hydraulic loading rate of 26 Lm⁻²min⁻¹ across the surface of the waste. This is roughly 25 times the design HLR of 1 Lm⁻²min⁻¹ used for related projects. As such, motor speed control was provided to reduce the flow rate of the pump down to a leachate flow of approximately 0.5 gpm. This hydraulic loading rate (1 Lm⁻²min⁻¹) is higher than the design HLR, but a safety factor is provided to ensure continuous flow. The selected low-voltage pumps are equipped with preinstalled dc brushed motors that may be controlled with voltage-based speed controllers (Control Resources, SmartFan Aurora DC Motor).
Speed Controller). The selected motor controllers receive an analog signal from the PLC control panel which allows remote monitoring and control of the pump speeds.

The selected pumps come from the manufacturer with a preinstalled pressure switch which will automatically disconnect power to the pump when the pressure at the outlet of the pump reaches 70 PSI. In the demonstration-scale system, this configuration protects the plumbing fixtures and piping from overpressure conditions in the event of a plugged fitting. The outlets of all three LBR pumps are connected to .5” rubber hoses which carry the leachate to the top of the LBRs where it is sprayed onto the substrate within the LBR.

**The Leach Bed Reactor**

The portable LBR modules (Fig. 32) are constructed from 60-gallon open head polypropylene drums which have threaded tank adapters installed into the lids and bases. The clearance required by the port in each base is provided by a metal drum carriage (or drum dolly) which raises the LBR off the ground. During operation, the LBR is connected into docking stations located within the body of the trailer unit.

![](image)

**Figure 32.** 60-gallon LBR

Leachate is pumped to the LBR and passes through a misting head (Dramm 10-12344 610F) which disperses the stream of water into a fine mist. This mist of liquid sprays out onto the surface of the substrate inside the LBR where it is further diffused as it begins to trickle through the unsaturated column. As the liquid passes through the bottom of the column, it passes through a non-woven monofilament geo-net composite material (Fig. 33). This material is of an unknown manufacturer as it was recovered from the surplus of another CSU project. This material serves to provide structural support for the waste substrate as well as to serve as a coarse filter for retaining solids entrained in the leachate stream.
The liquid which collects at the bottom of the LBR passes through a spiral mesh filter which serves to prevent particle sizes greater than 3 mm from passing into the LBR drainage line. This filter is constructed with a 3 mm plastic mesh wrapped in a spiral around a porous PVC pipe (Fig. 34). This filter is affixed to the drain bulkhead at the bottom of the LBR. A flexible hose carries the leachate through a series of valves and union disconnects before it drains into a common collection manifold.

**Leachate Collection and Leachate Return to LST**

The provided manifold is located underneath the trailer and has three drain ports to service three LBRs. The collection manifold is located underneath the floor of the trailer. Due to numerous layers of pipe insulation and heat trace cables, the manifold is obscured from view. The manifold serves to collect the leachate from the LBRs as well as direct the flow of the leachate to the sump pit. Embedded in ports in the manifold are temperature transmitters which are used to measure the temperature of the leachate exiting each LBR.

This manifold drains into a modular sump pit (Fig. 23). The elevation of liquid in the sump varies between 1.5’ and 4’ above the ground surface. A pump must be provided to return the liquid to the elevation of liquid inside the trailer, which is approximately 12’ above the ground surface. The sump pit is equipped with a sump pump, external level switches, and a drain port for use in system maintenance. The sump pump (Wayne Pump model ESP25) is a submersible pump operated on 12 V DC from an external circuit controlled by the PLC. The PLC uses feedback signals provided by two level switches (Grainger Item...
To determine when the sump pump should be operated. As liquid is pumped out of the pit, pressure is equalized in the pit with new leachate flows or gasses from the system. After the sump pump empties leachate from the sump pit, a check valve prevents liquid from draining back to the pit when the pump is not operating. As the leachate returns to the LST, it completes the first leg of the leachate recycle loop.

**Leachate Storage and Treatment System**

The leachate storage tank (300-gallon polyethylene tank) serves as a buffering tank for the leachate. In particular, this tank improves the operational characteristics of the FF. The methanogens within the FF are sensitive to small changes in the surrounding liquid’s solution chemistry. Thus, the LST acts as a buffering tank to allow concentrations of soluble compounds as well as the pH of the leachate to equalize before it is pumped to the FF. The decoupling of the hydrolysis stage from the methanogenic stage is essentially enabled by the ability to store leachate while still operating the LBR leachate pumps.

To remove the hydrolysis products produced by the LBR, the FF needs to be reliably supplied with leachate. The FF is equipped with a nearly identical leachate delivery process to the one the LBRs used. This leachate is pumped to the FF (300-gallon polyethylene tank; see details in section below) and then completes the cycle when it is returned to the LST.

**Leachate Pretreatment and Distribution**

The leachate pretreatment and associated leachate pump hardware for the FF is very similar to the hardware used for the LBR's filters and pumps (Fig. 35) (See LBR Leachate Pretreatment and Distribution and Leachate Pumps). The FF leachate supply equipment is physically located below the LBR equipment and follows a parallel and identical arrangement as described LBR Pretreatment section. These systems are distinct and completely separate but are linkable by a single valve placed at the common leachate manifold these two systems share (shown at bottom left of Fig. 28). The linked configuration allows leachate to be pretreated by either system and is utilized during filter maintenance as a provision to allow continued operation of the leachate supply pumps.
Leachate Pump

The fixed film reactor is equipped with a single pump identical to the pumps for the LBR system (Fig. 36). This pump, however, is not configured with a motor speed controller. Instead, it operates at its full capacity (2 gpm) whenever it is turned on. This pump delivers leachate to the top of the FF.

Fixed Film Reactor

The fixed film reactor uses suspended media to grow an attached film of anaerobic microbes, most notably methanogenic bacteria. Typical FF reactor installations used in industrial wastewater treatment consists of a FF in a single pass configuration. In such an installation the process liquid has a hydraulic retention time (HRT) within the reactor of no less than 24 hours. But unlike FFs used in single pass systems where HRT typically ranges from 30-72 hrs (Najafpour, 2006), the fixed film reactor in this system is configured in a recirculating loop where the effluent from the FF returns to the LST to be recycled again. This recycle loop enables the use of lower retention times than strict single pass systems. In a 24-hour period, the leachate will be recycled through the FF on
average 4.5 times, to spend on average 9 hours in the system (Fig. 23). The higher flow rate of the FF pump (120 gph) allows for higher velocities in the tank without the use of a dedicated FF recirculation pump as is often provided with FFs. While the HRT in the FF is low compared to typical anaerobic systems, of note is that the organic matter in the LST has already undergone hydrolysis in the LBRs, and some acidogenesis/acetogenesis has also likely occurred in the LST. Results confirmed that the 9-hour retention time was sufficient for conversion of organic matter in the LST to methane (see Experiment 5 Results). Once inside the FF, the pumped leachate partially fluidizes the media near the injection port at the top of the FF. The provided plastic media, BioPortz from Entex Technologies (37), is neutrally buoyant when suspended in water and readily fluidizes at low water velocities. This mechanism, although not fully utilized in this design, helps to free excess biological growth from the media, which helps the media maintain highly active biological films over its surface. The FF contains 30 ft$^3$ of BioPortz media. As the leachate migrates down through the FF, it comes in contact with the colonized surfaces of the media. Through this process, methanogens growing on the media convert soluble compounds within the leachate into methane and CO$_2$. Within the reactor, a concentration gradient is created from the top of the reactor towards the bottom. The concentration of organic molecules within the leachate progressively decreases as the liquid progresses through the media.

At the base of the FF, there is a large 2” threaded tank adapter that serves as a leachate exit port for the reactor. The leachate flows out through the port under gravity through a 2” diameter rubber hose which conveys the leachate under a very low-pressure loss back to the LST. Due to the hose size, the liquid level in the FF is only a fraction of an inch above the liquid in the LST when the FF leachate pump is operating. This transfer is a passive gravity flow transfer that serves as a simple liquid return.

Gas Handling System
The gas handling system serves as a conduit to extract biogas out of the reactors where it is generated so that it can be disposed of in a safe and effective manner. Low volumes of gas are produced from all parts of the system, and their removal from high points is
essential. The gas handling system also serves an important function in that it helps equalize gas pressures between tanks within the system.

**Gas Collection**

Every vessel where liquid levels change has a gas equalization line. Example vessels with this equalization line include the: LBRs, LST, FF, and sump pit (Fig. 23). In particular, these lines ensure a constant pressure in the head space for all tanks in the system. These gas lines also serve as a conduit for gas to be collected as it is produced in the system. The key gas production areas are the LST and FF reactors. Key gas equalization areas are the sump pit, LBRs, and gas storage tanks.

Gas collected from the system enters a common PVC pipe manifold physically located near the insulated roof of the trailer. This manifold is hidden from view for most of the extent of its length but serves an important role in conveying gasses and equalizing pressures throughout the system. This manifold has multiple PVC fittings into which the gas passes. These locations are equipped with isolation valves, sample valves, and/or union disconnects based on the requirements of each tank location. The mobile LBRs, for instance, have an isolation valve and a union disconnect to facilitate LBR isolation and removal at the end of the digestion process. Other locations such as the FF and LST are not equipped with a disconnect. Instead, they utilize valves at sample ports to provide access to sample gasses produced at those locations.

At the front of the reactor room, biogas collects in the gas manifold and flows toward the front of the trailer. The manifold ends at a “T” plumbing fitting that directs the flow either upwards or downwards. If gas pressure is above 0.5 psi, the biogas travels through a gas exit on the trailer’s roof.

In typical operations (< 0.5 psi gas pressure), the gasses travel downward and pass through a removable clear polycarbonate pipe that allows visual inspection of the gas stream. After this junction, the gas then is then directed towards the biogas storage tanks.

**Biogas Storage**

The biogas storage equipment used in this system is a collection of over water gas storage tanks (Fig. 38). This type of system uses gas pressure to lift a submerged tank out of a contained volume of water. The pressure inside the vessel can be modified by placing weights on top of the vessel and modifying the downward force. Under normal operations, gas pressure from the gas manifold causes the gas vessel to rise out of the water tank it is in, thus creating a variable volume constant pressure gas collection system. The water tank used for this application is a custom fabricated steel framed plywood tank coated with marine grade epoxy. The gas tanks used for this application are 1000 L blow molded polyethylene IBC tanks which have been inverted in the water tank.
Gas Disposal
Due to the combustible nature of the collected gas, it is important to ensure it is collected and removed from the interior space of the trailer. Under normal operations, the PLC initiates gas release to a provided stack. A level transmitter (Flowline EchoPod DL14) measures the height of the floating gas tanks at all times. When the measured level indicates that the tanks have reached 50% capacity (volume of stored gas approximately 1,000 L), a biogas solenoid valve (Asco 8200 series) located on the roof of the trailer is opened to enable the gas to escape through the biogas exit line out to the top of the flare stack (Fig. 39). Due to the low-pressure gas storage in this system (< 0.5 psi) the flare stack simply was a location for the gas to be released to atmosphere. An ignited flare would cause undue safety concerns.
Preparation and Loading of Substrate

Substrate Pre-processing

The substrate used to fill the LBRs for this project were of two types: pre-consumer food waste and yard waste (e.g. small limbs, leaves, etc.). In addition, the anaerobic inoculum was also added to LBRs in ratios based on the experiment plan outlined below (Anaerobic Inoculum Preparation). In a full-scale implementation of this technology, nearly the entire volume of the waste generated from a particular location would be processed in the system. At that scale, the substrate used in the process would inherently be representative of the available waste stream. In the scale of this experiment, however, only a small portion of the generated waste could be digested. As such, a careful approach was taken to select representative substrate samples for use in this study.

Because this study was conducted with such diverse substrates, it was imperative to use systematic methods to homogenize these substrates before loading into LBRs. This posed challenging in the demonstration-scale operations due to the larger volumes of substrate required. The detailed procedure for the collection and preparation of these three categories of waste substrates is explained in detail below.

Food Waste Collection

Pre-consumer food waste was collected from a variety of locations. During school months, food waste was collected from CSU’s housing and dining services as part of their established food waste diversion program. CSU dining facilities placed food waste products into 65-gallon dumpsters (Fig. 40). When food waste was not available from the University, pre-consumer wastes were collected from off-campus sources such as grocery
stores and coffee shops. Various food waste products were used in this study (Table 10). Of note, experimental batch 1 used a homogeneous waste of little variety, but efforts were taken to ensure representative samples in experimental batches 2-5.

![Figure 40. Food waste in 65-gallon waste container](image)

**Table 10.** Detail of the food waste substrates utilized in this experiment

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Waste Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1</td>
<td>Lettuce/ Kale/chopped greens</td>
</tr>
<tr>
<td>Batch 2</td>
<td>Chopped Raw Fruit Rinds, Salad Ingredients</td>
</tr>
<tr>
<td>Batch 3</td>
<td>Fruit, Pasta, Tomato Sauce</td>
</tr>
<tr>
<td>Batch 4</td>
<td>Fruit, Pasta, Tomato Sauce, Bread, Vegetables</td>
</tr>
<tr>
<td>Batch 5</td>
<td>Potato Wastes, Fruit, Coffee Grounds</td>
</tr>
</tbody>
</table>

**Food Waste Selection Criteria**

A suitable amount of waste, typically three 65 gallon dumpsters, were manually selected from the loading docks outside CSU dining facilities. The waste dumpsters were roughly categorized there and selected for use according to the criteria below:

1. Food waste was in an unprocessed state (i.e. pulped food waste would not be used).
2. > 90% of the waste could be categorized into definable basic categories (i.e. vegetables, pasta, meat).
3. Food type could be classified into no less than 3 basic categories.
4. Food waste was more or less made up of common food ingredients and was not disproportionately skewed by rare foods.
5. Wastes with standing liquid were excluded due to difficulties posed by the excess liquid to the collection of representative samples for analysis and use in the experiment.

There was often a wide variety of wastes to select from during these months. Thus, it was important to collect samples that would provide a good representation of the mix. For example, a 65-gallon dumpster with 45% kiwi fruit and 55% oranges would fail by criteria 2 and 4. Thus, a dumpster with these wastes in it would be passed over in favor of other containers. Based on this criteria, we selected only about 1 out of every 5 dumpsters that we inspected at the CSU dining facilities.

**Food Waste Preparation**

Once the food waste dumpsters were selected, they were loaded onto a hydraulic lift gate enabled truck and then transported to the digester site. The delivered food waste dumpsters needed to be composited so that each set of three reactors could be provided with the same representative substrate. This was accomplished by mixing the entire allocation of food waste used for the three 60-gallon LBRs in a shallow trough. An 110-gallon plastic stock tank (2’H x 3’W x 5’L) was used. Wastes from various sources were layered into the stock tank in such a way as to provide a partially homogeneous condition within the tank. This allotment of wastes was then manually inverted using shovels for several minutes. Once sufficiently mixed, this large batch of relatively homogenous food wastes served as a stockpile of waste to pull from during the final substrate preparation prior to loading of materials. A detailed outline of this is documented in below section (Substrate Blending).

**Yard Waste Material Collection**

A common source of yard wastes was collected in a single event from a local organics recycling company located in Fort Collins, CO. Wastes received at this facility were collected from around the city and spanned from grass clippings to woody biomass. Based on the criteria below, yard waste was selected from multiple piles of composting materials around the facility. A total of 12 cubic yards of yard waste was loaded into two truckloads and transported to the CSU laboratory.

**Yard Waste Material Selection Criteria**

Due to the considerable seasonal variation of yard wastes collected in our temperate climate, great care was taken to select and preserve a sample that was consistent and stable over time. Basic selection criteria for this material are listed below:

1) Roughly equal volumetric ratio of shredded wood chips, grass clippings, and tree leaves.
2) “Single grind” wood chips were selected and often contained small un-chunked branches and leaves.
3) A rough mix of both deciduous and coniferous wood chips and leaves were chosen.
4) Only very fresh lawn clippings were selected as to limit unnecessary decomposition prior to collection.

A representative sample of the collected yard waste was manually sorted into four distinct groups. The represented sample was first sieved with a 5 mm mesh to separate the larger particles from the smaller particles. The particles which passed through the sieve were categorized as the mixed particles fraction due to pragmatic considerations associated with separating and categorizing that material. The particles retained on the sieve (> 5 mm diameter) were manually separated with tweezers into three categories: wood chips, tree leaves and pine needles, and grass. These samples were oven dried at 110° C, and the resulting dry mass fractions of the ingredients in the yard waste were recorded Table 11).

<table>
<thead>
<tr>
<th>Categories of Substrates in Yard Waste Materials</th>
<th>Fraction by Dry Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood Chips &gt;5 mm</td>
<td>56.6 %</td>
</tr>
<tr>
<td>Tree Leaves and Pine Needles &gt;5 mm</td>
<td>10.2 %</td>
</tr>
<tr>
<td>Grass Clippings &gt;5 mm</td>
<td>12.2 %</td>
</tr>
<tr>
<td>Mixed Particles &lt;5 mm</td>
<td>20.8 %</td>
</tr>
</tbody>
</table>

Yard Waste Material Preparation
After delivery to our site, the truckloads were dumped out, mixed, and then carefully layered in a shallow pile approximately 15” deep on a large cement slab. The hot and dry weather allowed the surface of the pile to dry out considerably. After the surface of the pile had reached a dry consistency, the pile was then manually mixed in a bi-directional pattern. This mixing and drying process was repeated until excess moisture had been removed from the yard waste material. Afterward, the yard waste material was stacked into a 48” deep pile and covered with a tarp to protect it from moisture and extreme temperatures. It was stored for the duration of the experiment (five months). Total and volatile solids analysis of the yard waste samples were analyzed periodically to account for any degradation while in storage (See sections Solids Sampling Methods Total and Volatile Solids). Representative samples of yard waste were used as an additive to all LBR batches at 25% of the total mass (mass of solids and water) of each batch.

Anaerobic Inoculum Preparation
The initial digestate inoculum was collected from the acclimated inoculum utilized in the long-term laboratory-scale experiments conducted at 60% inoculum (see Laboratory-Scale Study Methods, Reactor Operation). Inoculum was delivered to the demonstration-scale system in a 5-gal bucket, purged with nitrogen and sealed. Due to the difference in scale between the laboratory-scale experiments and the demonstration-scale experiments, the 15 kg initial sample of inoculum was cultured in progressively larger
batches within the demonstration-scale LBRs. As each batch completed its digestion cycle, the resulting substrate was used as anaerobic inoculum for subsequent tests (Table 12). This inoculum production method mirrored the operation of the full system as it provided the inoculum fresh food waste and bulking materials at the beginning of the test, as well as continuous leachate delivery. All aspects of the inoculum grow-out procedure were operated in an analogous method to normal operations (see Substrate Blending, Inoculation Addition, and LBR Loading and Transport sections below for more details on these procedures).

Table 12. Outline of inoculum grow-out procedure

<table>
<thead>
<tr>
<th>Inoculation Batch</th>
<th># of LBRs Operated Simultaneously</th>
<th>Inoculum Wet Mass at Start (kg)</th>
<th>Food Waste to Inoculum Ratio</th>
<th>Inoculum Wet Mass at End (kg)</th>
<th>Inoculation Duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>15</td>
<td>2:3</td>
<td>30</td>
<td>21</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>30</td>
<td>2:3</td>
<td>50</td>
<td>21</td>
</tr>
<tr>
<td>C.1</td>
<td>2</td>
<td>40</td>
<td>2:3</td>
<td>75</td>
<td>21 (concurrent with 3.2)</td>
</tr>
<tr>
<td>C.2</td>
<td>2</td>
<td>5</td>
<td>10:1</td>
<td>50</td>
<td>21 (concurrent with 3.1)</td>
</tr>
</tbody>
</table>

The starter batch of inoculum at the beginning of the test was 15 kg and at the end of inoculation batches C.1 and C.2, this inoculum had been grown into a total of 125 kg of digestate ready for inoculation. This quantity of inoculum was enough to be used as inoculum for experimental batch 1. Similarly, the anaerobic digestate from previous experimental batches would be used as inoculum for all further experimental batch.

Substrate Blending

Once the food waste substrate, yard wastes substrate, and inoculum were properly processed as outlined above, these representative groupings were mixed together and added to the LBR. A primary goal in each experimental batch was to operate the LBRs with the same quantity of fresh food waste in each of the three replicates, and the maximum mass that could be added to an experimental batch was 60 kg. When a 60% inoculum LBR was tested (e.g., experimental batches 1-3), this meant adding 18 kg of food waste and varying amounts of inoculum and yard waste to achieve the desired percentage by mass of each material (Table 13). When the highest inoculation percentage tested was the 10% inoculum, a larger amount of food mass could be added to experimental batches (40.5 kg; Table 12). Yard waste was added so that it would make up 25% of the total mass of the prepared mass. For the most common total mass of 60 kg, this equates to 15 kg of yard waste. All masses for the substrate blending process
were measured as wet mass. The following outline specifies the systematic methodology used in the substrate blending process (Fig. 41 and 42).

1. An 110-gallon stock tank was placed on a low-profile floor scale, 5,000 lb, (U-line brand)
2. The scale was tared to the weight of the empty 110-gallon tank.
3. The required mass (25 % of total mass) of yard waste was added to the bin (Table 13).
4. The specified amount of food waste was added to the bin (Table 13).
5. The material was mixed thoroughly until the food waste and yard wastes were fully incorporated. Particular attention was paid to the bottom and corners of the tank.

![Figure 41](image1.png)

**Figure 41.** 110 Gallon mixing tank. Note blue pallet scale which is used to measure the substrates mass.

![Figure 42](image2.png)

**Figure 42.** 110 Gallon mixing tank with food waste added on top of wood chips
Table 13. Substrate ratios used in this experiment

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Replicate Number</th>
<th>Inoculation (%)</th>
<th>Food Waste Wet Mass (kg)</th>
<th>Yard Waste Wet Mass (kg)</th>
<th>Inoculum Wet Mass (kg)</th>
<th>Total Wet Mass (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1</td>
<td>R1.1</td>
<td>60%</td>
<td>18</td>
<td>15.0</td>
<td>27.0</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>R1.2</td>
<td>10%</td>
<td>18</td>
<td>6.7</td>
<td>2.0</td>
<td>26.7</td>
</tr>
<tr>
<td></td>
<td>R1.3</td>
<td>-</td>
<td>18</td>
<td>6.0</td>
<td>-</td>
<td>24.0</td>
</tr>
<tr>
<td>Batch 2</td>
<td>R2.1</td>
<td>60%</td>
<td>18</td>
<td>15.0</td>
<td>27.0</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>R2.2</td>
<td>10%</td>
<td>18</td>
<td>6.7</td>
<td>2.0</td>
<td>26.7</td>
</tr>
<tr>
<td></td>
<td>R2.3</td>
<td>-</td>
<td>18</td>
<td>6.0</td>
<td>-</td>
<td>24.0</td>
</tr>
<tr>
<td>Batch 3</td>
<td>R3.1</td>
<td>60%</td>
<td>18</td>
<td>15.0</td>
<td>27.0</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>R3.2</td>
<td>10%</td>
<td>18</td>
<td>6.7</td>
<td>2.0</td>
<td>26.7</td>
</tr>
<tr>
<td></td>
<td>R3.3</td>
<td>-</td>
<td>18</td>
<td>6.0</td>
<td>-</td>
<td>24.0</td>
</tr>
<tr>
<td>Batch 4</td>
<td>R4.1</td>
<td>10%</td>
<td>40.5</td>
<td>15.0</td>
<td>4.5</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>R4.2</td>
<td>10%</td>
<td>40.5</td>
<td>13.5</td>
<td>4.5*</td>
<td>54.0*</td>
</tr>
<tr>
<td></td>
<td>R4.3</td>
<td>-</td>
<td>40.5</td>
<td>13.5</td>
<td>-</td>
<td>54.0</td>
</tr>
<tr>
<td>Batch 5</td>
<td>R5.1</td>
<td>10%</td>
<td>40.5</td>
<td>15.0</td>
<td>4.5</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>R5.2</td>
<td>10%</td>
<td>40.5</td>
<td>15.0</td>
<td>4.5</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>R5.3</td>
<td>-</td>
<td>40.5</td>
<td>13.5</td>
<td>-</td>
<td>54.0</td>
</tr>
</tbody>
</table>

*(R4.2 was inoculated with the injected inoculation method so its prepared mass is 4.5 kg less than R4.1)*

Inoculation Addition
The three inoculation methods (mixed, injected and top; Table 4) were evaluated in the six experimental batches of the demonstration-scale MSAD. The solid digestate collected from the anaerobic inoculum growth batches C.1 and C.2 were used as inoculum for LBR experimental batch 1. Inoculum from all subsequent experimental batches was derived from the previous experimental batches. In experimental batches 1-3, inoculum was added to new LBR batches at 0 %, 10 %, and 60 % ratios by wet mass (Table 13). In subsequent experimental batches (4-5), enhanced leachate delivery methods were evaluated (injected and top inoculation; Table 4).

Mixed Inoculum Methodology
Steps 1-4 were followed as described in Substrate Blending. For LBR batches that required a mixed anaerobic inoculum, the appropriate amount of inoculum was then added to the top of the mix inside the 110-gallon tank. Then the entire batch was then mixed until the inoculum was fully incorporated. After being fully mixed, the material was ready for reactor loading (see LBR Loading and Transport). All three LBRs were delivered leachate from the same source, and thus the impact of leachate inoculation should have been
similar for all LBRs. These three inoculum ratios were tested in parallel using the same substrates.

**Injected Inoculum Methodology**

In the inoculation injection method, the inoculum was placed in a 20 liter sealed vessel where leachate was passed prior to being pumped into LBR 1 (Fig. 43). Within this vessel, leachate flowed around submerged particles of solid digestate that were contained within a coarse mesh bag composed of a French drain mesh tube which had been closed at both ends (Advanced Drainage Systems 0420HA). This method was used only for LBR 1 of experimental batch 4 (R4.1) (Table 4).

![Inoculum injection process diagram](image)

**Figure 43. Inoculum injection process diagram**

**Top Inoculated- Inoculum Methodology**

Steps 1-4 were followed as above. In experimental batch 5 (Table 13), the top inoculation approach was utilized for LBR 1. In this method, the LBR was filled in the same fashion as a non-inoculated column. However, before the lid was sealed on the LBR, the entire mass of the inoculum (in this case 4.5 kg, Table 13) was added to the top of the LBR. At the start of the batch, liquid leachate was trickled through the top inoculum with the hope that liquid leachate would transport the inoculum throughout the LBR.

**LBR Loading and Transport**

LBRs were filled on the ground level and then lifted onto the dock with the barrel elevator. The LBRs were then transferred into the interior of the trailer where they were connected into the leachate and gas plumbing of the trailer.

1. The entire volume of the mix was added into the LBRs with efforts taken to limit the time the mix was exposed to air.
2. An excess sample of approximately 20 L was removed for solids analysis (Solids Sampling Methods).
3. The lid was then placed on the LBR and was sealed with the factory provided drum seal.
4. The LBR was sealed and transported to the loading dock where it was loaded on the drum elevator (Fig. 44).
5. The barrel elevator was used to transfer the drum from the loading dock to ground level without the use of heavy equipment.
6. Once the LBR was placed on the loading dock, it was rolled inside and connected to one of the three loading docks.

![Drum elevator next to loading dock](image)

**Figure 44.** Drum elevator next to loading dock

**Analytical Methods**

The multiple objectives of this experiment required the ability to measure the results of the various experimental batches conducted. These results were primarily measured through analysis of solid samples taken from the different substrates and inoculums, as well as liquid samples of the leachate from various points in the system.

**Solids Sampling Methods**

As outlined above, the substrates utilized in this study were highly variable in size, shape, and composition. In this experiment, total and volatile solids analysis were the two primary methods for characterizing the solid substrates and inoculums. All solid substrates, inoculums, and the resulting digestate were analyzed independently. After these materials were blended together and before they were added to the LBRs, another sample was collected. Both analyses were based on a 10-15 g sample weight. With the above highly variable substrates, it was very challenging to collect a representative sample of only 10-15 g. As such, a rigorous and systematic sample collection methodology was developed. This methodology was a multi-step process that used a 212
cc wood chipper (Earthquake brand; Fig. 45) to pulverize batches of 20 L of materials at a time. Before the start-up of the three LBRs, 20 L representative samples (collected from more than 4 locations of each material) were taken from the unblended food waste, composted yard waste, and the post-digestate anaerobic inoculum. In addition to the individual samples, the blended mix (see section Substrate Blending) was also sampled for each LBR that was prepared.

Each of these 20 L samples was then passed through the chipper and the resulting pulverized samples collected for solids analysis. Each batch was pulverized with the waste shredder (Fig. 45) to produce a pulped material. The pulped material was collected in a 5-gallon bucket and manually mixed. Three to five samples of 10-15 g each were collected for solids analysis from each 5-gallon bucket.

**Figure 45.** Waste shredder with 4” PVC nozzle installed on the outlet

### Total and Volatile Solids

EPA Method 1684 was used to analyze the total solids (TS), fixed solids (FS) and volatile solids (VS) of the substrates and inoculums employed in this test. All solid waste products processed as described above (Solids Sampling Methods) were analyzed for solids content. This provided information about the pre- and post-digestion TS and VS percentages. Experimental batches 1-4 were compared as the difference between the initial and final VS. The final % VS value was subtracted from the initial % VS value to yield the decrease in % VS. **This method of comparing LBR performance was found to be limited in its application due to leaching of fixed and volatile solids from the solids mass into the leachate.**

Due to limitations in data collected for experimental batches 1-4, the analysis utilized for experimental batch 5 used the initial and final TS and VS percentages by multiplying them
by the total mass of the substrate (i.e. wet mass; $m_{ws}$) in the reactor at the respective sampling points (Eqn. 1 and 2). This yielded the quantity of the entire mass of the TS and VS at the beginning and the end of the experiment. The mass of volatile solids $m_{vs}$ within the reactor was determined at the beginning and end of experimental batch 5 ($m_{vs\text{ initial}}$ and $m_{vs\text{ final}}$). These values were used to calculate the removal efficiency for VS ($\%VS_{reduction}$) over the duration of the experiment (Eqn. 3).

$$\%TS \times m_{ws} = m_{ts} \quad \text{(Eq. 1)}$$
$$\%VS \times m_{ts} = m_{vs} \quad \text{(Eq. 2)}$$
$$\%VS_{reduction} = \left(\frac{m_{vs\text{ initial}} - m_{vs\text{ final}}}{m_{vs\text{ initial}}}\right) \quad \text{(Eq. 3)}$$

It is important to note that the decrease in $\%VS$ reported for Experiments 1-4 is different from the $\%VS_{reduction}$ reported for Experiment 5. Further, reported $\%VS_{reduction}$ is the same metric reported for the laboratory-scale studies.

**Leachate Sampling Methods**

It had become clear after analysis of the previous four experimental batches that additional information was needed to augment the solids analysis from these experiments. The use of TS and VS analysis methods was complicated by the utilization of the relatively recalcitrant yard waste. In addition to the solids analysis employed in the first four experiments (experimental batches 1-4), leachate samples were also collected and analyzed from the fifth experiment (experimental batch 5). These leachate samples enabled a complete characterization of the process. In particular, leachate analysis was conducted over the duration of the study, which helped to augment the TS and VS data which was only collected at the beginning and the end.

In this process, leachate serves as a primary transport mechanism in the flow of organic compounds through the system. This created an opportunity for collecting detailed data on the production, destruction, and accumulation of organic compounds within the leachate.

In the 5th experimental batch, leachate was collected between two and three times per week (greater frequency at the beginning of the test) from multiple points in the interconnected system. The primary points for leachate collection were at the leachate pump manifold (Fig. 36) and at the leachate drain port located at the base of each individual LBR (Fig. 23). The samples collected from these points effectively resulted in leachate being collected before it entered and after it exited the LBR. This sampling configuration enables analysis of the flow of organic compounds in and out of the LBR.

Multiple tests were conducted on the leachate including chemical oxygen demand (COD), biochemical methane potential (BMP), conductivity, and total nitrogen. The COD test was performed as a method of quickly estimating the overall concentration of oxidizable compounds within the leachate. BMP tests were carried out to understand the
anaerobic degradability of these leachates. The ratio of BMP to COD was used to estimate the relative production of methane producing compounds from the LBR using the entire set of COD data and a correlation between COD and BMP that was established via multiple sampling events (see BMP Estimation Section below). Conductivity and total nitrogen were collected to compare the conducted experiments to previous work funded by this grant. Due to the recirculating nature of this process (and the conservative nature of ionic solutes and ammonia), these tests were only conducted on a limited number of LST samples.

**Chemical Oxygen Demand Samples**

Leachate COD samples were analyzed by using Hach high range (20-1,500 mg/L) Test ‘N Tube COD test kits. These tests were conducted weekly on samples which were immediately frozen after collection.

**Biochemical Methane Potential**

BMP tests were utilized in this experiment as a way of determining the methane producing fraction of the COD at different periods of the cycle. An anaerobic inoculum and a nutrient solution were used in conjunction with a suitable organic substrate to enable the formation of biogas. The biogas is collected and analyzed for methane content. In the CSU MSAD system, these tests help determine the gas production potential of the leachate as it exits the LBR.

The BMP tests we conducted in 150 ml Luer lock syringes by a previously developed method (Quinn 2014). To each syringe, 50 ml of a liquid nutrient solution and 25 ml digester inoculum were added. Then 100 mg of COD worth of leachate was added (typically between 5-20 ml leachate). Gas volumes were recorded daily, and the methane content of the gas was analyzed using a Hewlett Packard Series 2180 gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with an Alltech column (Alltech, Deerfield, IL) packed with HayeSep Q 80/100 mesh (HayeSeparation, Inc., Bandera, TX). The gas chromatograph was operated at injection and detector temperatures of 100°C.

**Data Analysis Methods**

**Substrate Normalization**

Yard waste substrates composed 25% of the total wet mass of the digestion substrate. The lignocellulosic compounds in the woody biomass within the yard waste is poorly biodegradable under the anaerobic conditions within this process. Thus, the lower degradability of these samples suppressed the apparent degradation of the food waste within the process as measured by volatile solids. However, there was no clear way to specify the difference between the yard waste and the food waste at the end of the process. For example, if a significant amount of volatile solids reduction was achieved in the food waste substrate a simple measurement of volatile solids in the post-digestate may not reveal volatile solids reduction due to the remaining fraction of woody biomass that still contains volatile solids. The interpretation of the volatile solids destruction in the
process was normalized based on literature values for the % biodegradability of the various fractions of the yard waste. These literature values were based on a large set of data which sought to determine the biodegradability of various substrates under anaerobic conditions. (Triolo, 2012)

The method used in this analysis used a literature provided value for the fraction of each substrates VS which was composed of cellulose ($\%VS_{Cellulose}$). This value was then multiplied by that fractions biodegradable cellulose fraction ($\%BD_{Cellulose}$), provided by the same force, to yield the biodegradable fraction of VS ($\%BD_{VS}$) (Eqn. 3). The biodegradable VS fraction value is then multiplied by the mass of volatile solids for the experiment to yield the mass of biodegradable solids ($\%BD_{VS}$) for each of various fractions of the yard waste (Triolo, 2012). The fraction of non-degradable solids within the yard waste represented the mass of yard waste which would be left unchanged in the process. This mass of the non-degradable yard waste was subtracted from the mass VS value in Eqn. 2. Using this approach, the removal percentages for the food waste values were more easily separated from the more recalcitrant lignocellulosic wastes. Future studies may include an in-house study of biodegradability, specific to the substrates included in experiments, instead of literature provided value.

<table>
<thead>
<tr>
<th>Substrate Type</th>
<th>Cellulose Fraction of VS in Substrate ($%VS_{Cellulose}$)</th>
<th>Biodegradable Cellulose Fraction ($%BD_{Cellulose}$)</th>
<th>Biodegradable VS Fraction ($%BD_{VS}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lawn Cuttings</td>
<td>47.5%</td>
<td>66.6%</td>
<td>31.6%</td>
</tr>
<tr>
<td>Hedge Cuttings (with leaves)</td>
<td>42.0%</td>
<td>39.9%</td>
<td>16.8%</td>
</tr>
<tr>
<td>Wood Cuttings</td>
<td>45.0%</td>
<td>32.7%</td>
<td>14.7%</td>
</tr>
</tbody>
</table>

The non-degradable portions of this material were regarded as inert and removed from the solids calculations noted above (Total and Volatile Solids).

\[
\%VS_{Cellulose} \times \%BD_{Cellulose} = \%BD_{VS} \quad \text{(Eqn. 3)} \\
\%BD_{VS} \times m_{VS} = m_{bdVS} \quad \text{(Eqn. 4)}
\]

**BMP Estimation**

BMP serves as an important analysis to understand the methane potential of the leachate generated from this multi-staged technology. Yet the analysis is costly and time-consuming. We sought to use the lower cost and quick results from COD analysis as an analog for the BMP value. By analyzing both COD and BMP for many samples, a ratio of BMP to COD was found. This ratio was then applied to the larger set of COD data to estimate the BMP of the leachate at all points COD was collected (Table 15).
Table 15. Summary of BMP:COD ratios for the BMP estimation procedure (LST samples)

<table>
<thead>
<tr>
<th>Day of Test</th>
<th>Measured BMP:COD Ratio</th>
<th>Applied BMP:COD Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.140</td>
<td>0.140</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>0.140</td>
</tr>
<tr>
<td>12</td>
<td>0.018</td>
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<td>0.018</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>0.018</td>
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<tr>
<td>23</td>
<td></td>
<td>0.018</td>
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<tr>
<td>33</td>
<td>0.007</td>
<td>0.007</td>
</tr>
<tr>
<td>37</td>
<td></td>
<td>0.007</td>
</tr>
</tbody>
</table>
Acknowledgements

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References