# ABSTRACT

LOWER, LILLIAN MARIE. Thermophilic Anaerobic Digestion of Lemnaceae Biomass for Biogas Production. (Under the direction of Drs. Jay Cheng and Joe Sagues).

Anaerobic digestion of sustainably sourced biomass to generate biogas is a vital form of renewable energy that provides significant benefits to the environment. Biogas production from the anaerobic digestion of conventional feedstocks, including agricultural and municipal wastes, is inadequate when considering growing energy demands. Alternatively, the dedicated use of sustainably grown, non-food crops for biogas production has attracted attention in the last decade. An aquatic plant family, Lemnaceae, commonly known as duckweeds, has shown great potential as a next generation biomass feedstock for anaerobic digestion that is decoupled from arable land use. Lemnaceae plants grow incredibly fast and can be continuously harvested, making them an ideal candidate for bioenergy production. Knowledge gaps exist regarding the thermophilic anaerobic digestion of Lemnaceae biomass. The study aims to answer key questions regarding this process by completing two key objectives: I. Develop a continuous thermophilic anaerobic digestion system processing three duckweed varieties and swine wastewater. II. Perform batch testing using the biomass to ascertain the biomethane potential of the biomass and estimate parameters associated with the kinetics of the digestion process. Continuous digestion was performed at 50 ºC with a 10 day hydraulic retention time at four organic loading rates: 1,000, 2,000, 3,000, and 4,500 mg COD $L^{-1}$ digester $day^{-1}$. Methane yields increased with the addition of duckweed and increasing organic loading rate with a maximum yield of 0.362 $m^3$ $CH_4$ $kg^{-1}$ $COD_{consumed}$ produced by the reactor processing the *Spirodela* duckweed type. Batch testing produced results in agreement with methane production rates determined from the continuous system. The three duckweed types did not have significantly different methane productions in terms of $COD_{added}$ and the average biomethane potential (BMP)

was 0.184 $m^3$ $CH_4$ $kg^{-1}$ $COD_{consumed}$. First order and transference kinetic models fit well to the experimental data and estimated parameters for methane production, maximum methane production rate, and the hydrolysis constant. Future research regarding thermophilic anaerobic digestion of duckweed biomass should focus on the microbial community structure of the anaerobic microbiome.

# Thermophilic Anaerobic Digestion of Lemnaceae Biomass for Biogas Production

by
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Lillian Lower was born and raised in Casper, Wyoming. She attended Gonzaga University in Spokane, WA where she studied Civil Engineering and Mathematics. After earning her bachelor's degree in 2020 she served as an AmeriCorps volunteer in Wheeling, West Virginia working with a non-profit urban agriculture organization called Grow Ohio Valley. She then joined the Wolfpack at North Carolina State University in 2021 to pursue her master's degree in Biological and Agricultural Engineering under the guidance of Dr. Jay Cheng and Dr. Joe Sagues. She will continue her studies in BAE at NCSU and pursue her PhD under the guidance of Dr. Joe Sagues.

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## 1.1. Introduction

Biogas, compared to other alternative biofuels (e.g. biodiesel, bioethanol, biobutanol) has a better ratio of energy produced to energy required for production and lower life cycle emissions (Rouches et al., 2016). A common mechanism for biogas production is the anaerobic digestion of biomass by microorganisms. Anaerobic digestion of sustainably sourced biomass to generate biogas is a vital form of renewable energy that provides significant benefits to the environment. Conventionally, feedstocks for anaerobic digestion have included agricultural and municipal wastes, and these systems have been well-researched with many of them applied in industrial operations (Mutungwazi et al., 2021). However, biogas production from conventional feedstocks faces challenges including affordability, availability, efficiency, and sustainability (Calicioglu et al., 2018). Alternatively, the dedicated use of sustainably grown, non-food crops for biogas production has attracted attention in the last decade. The availability of arable land to sustainably grow crops dedicated for biogas production is severely limited, and thus unconventional biomass feedstocks must be developed for anaerobic digestion. The aquatic plant family, Lemnaceae, commonly known as duckweeds (Figure 1.1.), have shown great potential as a next generation biomass feedstock for anaerobic digestion that is decoupled from land use (Yadav et al., 2017).

**Figure 1.1.** *Lemna Gibba* (Sicily), a variety of duckweed that supports frond growth of 1/8”-3/16”

Duckweed is a small free-floating aquatic plant (Figure 1) that is classified into five genera: *Landoltia*, *Lemna*, *Spirodela*, *Wolffia,* and *Wolffiella* (Liu et al., 2021). It is an extremely adaptable plant that has been studied for its nutrient uptake and wastewater treatment abilities (Nahar and Sunny, 2020). Duckweed has been evaluated as a feedstock for bioenergy production for several decades. While there has been success with duckweed-based bioethanol production via fermentation, anaerobic digestion of the plant has demonstrated more feasibility as a biofuel production technique (Calicioglu et al., 2021). Duckweed is an ideal feedstock for anaerobic digestion for many reasons (Table 1.1.). A key reason is that duckweed, unlike many other conventional terrestrial biomasses for anaerobic digestion, does not compete with food or feed production.

**Table 1.1.** Bioenergy feedstock advantages and disadvantages.

| **Trait** | **Duckweed** | **Terrestrial Plant** |
|---|---|---|
| Rapid growth | ✓ | |
| Higher biomass per plant | | ✓ |
| No competition with crops for arable land | ✓ | |
| Lower water usage | ✓ | |
| Lower energy-consuming cultivation | ✓ | |
| Storing $CO_2$ from atmosphere | ✓ | ✓ |
| Effortless harvesting | ✓ | ✓ |
| Lower biorefinery cost | ✓ | |

This review presents recent literature on duckweed as a feedstock for anaerobic digestion and discusses how key characteristics of the plant make it an ideal candidate for biogas production. The characteristics of duckweed selected for this discussion are the growth, composition and nutrient recovery abilities of the plant. These characteristics will be connected to previous research reviewed regarding biogas production from duckweed biomass.

### 1.2. Growth

Duckweed has a small simple structure and is one of the fastest growing plants in the world (Liu et al., 2021). It achieves very fast growth rates by reproducing asexually from mother fronds (Pena et al., 2017). While duckweed is naturally occurring, and capable of dominating natural waterbodies, it requires cultivation in nutrient-rich water under optimized conditions to reach maximum growth potentials. In engineered growing systems, the nutrient-rich water can be agricultural, municipal, or industrial wastewaters, so the plant does not depend on manufactured fertilizers (Pena et al., 2017). Bergmann et al. (2000b) demonstrated the necessity of establishing optimized growing conditions for duckweed biomass production and wastewater treatment (Bergmann et al., 2000b). Within their study, swine lagoon effluent diluted at 50% supported the healthiest biomass production of *Lemna gibba* 8678 and *Lemna minor* 8627 while maintaining steady nutrient removal. However, it was noted that effluent concentrations for ideal production will vary depending on geographic isolate selected and effluent composition. While each reviewed study of the plant used different growing conditions, Table 1.2. lists several duckweed varieties and maximum growth rates achieved. Cui and Cheng (2015) optimized growing conditions to report one of the fastest duckweed growth rates with a 16-24 hour doubling time (Cui and Cheng, 2015).

**Table 1.2.** Duckweed maximum growth rates resulting from a literature review evaluating optimized duckweed production systems.

| **Duckweed Species** | **Growth Rate** | **Author** |
|---|---|---|
| *Lemna minor* | 3.4 g dry weight $m^{-2}$ $day^{-1}$ | Pena et al. (2017) |
| *Spirodela punctata 7776, Lemna minor 8627, & Lemna gibba 8678* | 30 g dry weight $m^{-2}$ $day^{-1}$ | Cheng and Stomp (2009) |
| *Landoltia S3* | 30.35 g dry weight $m^{-2}$ $day^{-1}$ | Xiao et al. (2013) |
| *Spirodela punctata 7776* | 31.92 g dry weight $m^{-2}$ $day^{-1}$ | Cheng et al. (2002) |
| *Spirodela polyhiza* | 995 g wet weight $m^{-2}$ $day^{-1}$ | Matošević et al. (2019) |
| *Spirodela polyrhiza*<br>*Lemna minor*<br>*Lemna gibba*<br>*Lemna punctata* | 70.3 g dry weight $m^{-2}$ $day^{-1}$<br>42.2 g dry weight $m^{-2}$ $day^{-1}$<br>46.1 g dry weight $m^{-2}$ $day^{-1}$<br>48.6 g dry weight $m^{-2}$ $day^{-1}$ | Toyama et al. (2018) |
| *Landoltia punctata* | 5.72 g dry weight $m^{-2}$ $day^{-1}$<br>3.27 g dry weight $m^{-2}$ $day^{-1}$ | Tonon et al. (2017) |

Duckweed growth rate optimization is a necessary step for any study using a unique species in designed growing conditions. This optimization is not conducted by the use of fertilizer, as it is with many crops, but with nutrients provided by wastewaters. Regardless of duckweed species, the initial stage of growth, from seed or transplanted fronds, is the rate-limiting, or lag, phase. Cheng et al. (2002) suggested that slowly acclimating the plant to the nutrient-rich liquid may accelerate initial growth and reduce the lag phase (Cheng et al., 2002).

This study used both outdoor and indoor tanks for propagation, and determined that the plants were moderately sensitive to temperature and light fluctuations. Duckweed growth rates increased with longer periods of higher light intensity and warmer temperatures than a natural cyclical climate (Cheng et al., 2002). Liu et al. (2021) conducted a review of available research evaluating aquatic phytoremediation via duckweed cultivation (Liu et al., 2021). They determined that microorganisms, growing in symbiosis with duckweed, support growth of the plant and increase resilience. There is a need for research investigating duckweed-specific plant growth-promoting bacteria.

Unlike many conventional energy crops and agricultural residues used for anaerobic digestion, duckweed grows continuously and can be harvested continuously (Cui and Cheng, 2015). This can be extrapolated into an annual production rate of 106 dry tonnes of duckweed per hectare under optimal growing conditions. This value clearly surpasses the annual production rate of corn, calculated to be 7.84 tonnes per hectare (Cui and Cheng, 2015). The rapid growth of duckweed is a key characteristic that enables constant energy production in a biogas-generating system.

### 1.3. Composition

A prominent challenge for the anaerobic digestion of agricultural residues and most energy crops, is the slow degradation of lignocellulosic biomasses (Xu et al., 2019). Biomass recalcitrance, or resistance to hydrolysis due to stubborn composition, leads to low bioconversion efficiencies and lengthy, expensive pretreatment requirements. This is not a challenge facing anaerobic digestion of duckweed biomass because the plant lacks a recalcitrant cell wall (Tonon et al., 2017). The simple composition of duckweed theoretically requires no pretreatments and enables simple energy production via anaerobic digestion or ethanol fermentation (Yadav et al., 2017). By altering the growing conditions of duckweed, this composition can be altered to better accommodate the feedstock's purpose. A principal example of this manipulation is nutrient starvation for increased starch production (Verma and Suthar, 2015). This review will discuss the composition and structure of duckweed biomass, focusing on lignocellulosic, proximate, and ultimate compositions, and elucidate how composition trends reflect an ideal biomass for biogas production.

Duckweed contains mostly proteins, starch, cellulose, and hemicellulose (Cheng and Stomp, 2009). Duckweed has little lignin. Yadav et al. (2017) conducted a comparative study looking at duckweed and cattle dung as prospective feedstocks for anaerobic digestion. From their lignocellulosic content analyses of the two feedstocks, duckweed fiber was composed of 55% cellulose, 33% hemicellulose, and 12% lignin. Duckweed contains roughly 6% more cellulose and 56% less lignin than cattle dung (Yadav et al., 2017). These values are important because more cellulose reflects more available sugars, and less lignin translated to greater degradability of the biomass. Calicioglu and Brennan (2018) reported duckweed compositions of 17% cellulose, 18-24% hemicellulose, 4-16% starch, 17-26% crude protein, and 1-2% lignin (Calicioglu and Brennan, 2018). The key factor of this composition is the low lignin. Low lignin concentrations enabled fast hydrolysis of the feedstock by microorganisms in anaerobic digestion. For comparison, corn stover, a common agricultural residue used for bioenergy production, contains roughly 11% lignin and often requires pretreatment prior to anaerobic digestion (Hashemi et al., 2021).

One may argue that biomass such as corn accounts for the energy required for pretreatment by producing high quantities of starch which can be processed into biofuels. However, Cheng and Stomp (2009) observed that duckweed could be optimized to produce five times the amount of starch per hectare per year that corn could produce (Cheng and Stomp, 2009). Duckweed-to-ethanol production also required less energy than corn-to-ethanol, which resulted in lower costs across the production and processing system. Cui and Cheng (2015) reviewed and discussed potential techniques that altered the composition of duckweed for increased starch concentrations (Cui and Cheng, 2015). Starch in duckweed is generated by photosynthesis, which is difficult and expensive to stimulate. As opposed to increasing starch generation, prevention of starch deterioration was determined to be more economically and technically feasible. To prevent starch degradation in duckweed, growing conditions were proposed that reduced starch utilization and increased accumulation. Successful methods included nutrient starvation, temperature reduction, increased cation exposure and the addition of chemical inhibitors (Cui and Cheng, 2015). Nutrient starvation echoed throughout the literature as a method for increased biomass quality and starch content (Verma and Suthar, 2015). While these methods increased bioethanol and biobutanol production, the implication of these practices in anaerobic digestion for biogas production was not outlined. It can be deduced, however, that increasing starch concentrations in duckweed would not increase the difficulty of microbial hydrolysis or anaerobic digestion.

Proximate and ultimate analyses are critical for the evaluation of a biomass for bioenergy production as they outline the biomass’s energy potential (Verma and Suthar, 2015). Many studies have conducted proximate analyses of duckweed species and compared them to other common feedstocks for bioenergy production (Table 3). The components that will be discussed within this review are moisture, ash, and volatile matter contents. While anaerobic digestion is suitable for treating feedstocks with high moisture contents, water is not an nutrient-rich food source for the anaerobic digestion microbiome (Tabatabaei et al., 2020). In each study summarized in Table 1.3., duckweed was dried using sunlight. However, the outlier of 96% moisture reported by Buragohain et al. (2021) is not entirely consistent with the other studies. Noting this, it is difficult to make comparisons or assumptions across these studied with regards to moisture content. Higher ash contents represent higher likelihood of the biomass to contribute to hydrolytic resistance within the anaerobic digestion process (Gaur et al., 2017). The ash content of the duckweed species listed below was comparable to that of the other waste, agricultural, or energy crop materials. Volatile matter is an incredibly important component of biomass for anaerobic digestion as it represents hydrocarbons and organic acids that will be consumed by methanogens (Schnaars, 2012). Higher amounts of volatile matter suggests better suitability for hydrolysis, enzymatic degradation, and higher methane outputs (Gaur and Suthar, 2017). In the studies reviewed, duckweed supported higher volatile matter contents than that of cattle dung and waste activated sludge (Yadav et al., 2017; Gaur et al., 2017). Within these studies, duckweed was a more productive feedstock for the microbial community.

**Table 1.3.** Compilation of reviewed proximate analyses of duckweed varieties and common anaerobic digestion feedstocks.

| **Biomass** | **Moisture (%)** | **Ash (%)** | **Volatile matter (%)** | **Fixed carbon (%)** | **Reference Literature** |
|---|---|---|---|---|---|
| *Lemna gibba* | 10 | 13 | 64 | 15 | Gaur et al. (2017) |
| Unspecified Duckweed (UD) | 12 | 4 | 84 | 0.3 | Yadav et al. (2017) |
| *Spirodella polyrrhiza* | 4 | 19 | 57 | 20 | Gaur & Suthar (2017) |
| *Lemna minor* | 96 | 16 | 75 | 10 | Buragohain et al. (2021) |
| Waste Activated Sludge (WAS) | 77 | 4 | 25 | 5 | Gaur et al. (2017) |
| Cattle Dung | 14 | 14 | 62 | 10 | Yadav et al. (2017) |
| Switch grass | 63 | 5 | 79 | 16 | Buragohain et al. (2021) |
| Rice straw | 12 | 13 | 75 | 12 | Buragohain et al. (2021) |

The ultimate analysis of prospective feedstocks for anaerobic digestion (Table 1.4.) is not only important for overall energy potential, but also for the calculation of the feedstock's carbon/nitrogen (C/N) ratio within the digester. A stable and productive anaerobic digester has a C/N ratio of around 25:1, respectively (Yadav et al., 2017). From Table 1.4, it can be concluded that duckweed often supports lower C/N ratios than competing feedstocks and lower than optimal C/N ratios than what is ideal for biogas generation. The lower C/N ratio of duckweed could result in an excess of nitrogen with respect to carbon. This excess results in less methane produced and higher ammonium concentrations, which can be toxic to the bacteria in the anaerobic digestion process (Yadav et al., 2017). The nitrogen-heavy composition of duckweed makes it an ideal candidate for co-digestion with materials that have a higher C/N ratio, for example, food or agricultural waste materials (Navarro et al., 2022).

**Table 1.4.** Compilation of reviewed ultimate analyses of duckweed varieties and common feedstocks for anaerobic digestion.

| **Feedstock** | **Carbon (%)** | **Hydrogen (%)** | **Nitrogen (%)** | **C/N ratio** | **Reference Literature** |
|---|---|---|---|---|---|
| *Lemna gibba* | 42 | 4 | 4 | 10.20 | Gaur et al. (2017) |
| Unspecified | 47 | 6 | 5 | 9.50 | Yadav et al. (2017) |
| *Spirodella polyrrhiza* | 33 | 4 | 2 | 16.50 | Gaur & Suthar (2017) |
| *Lemna minor* | 31 | 4 | 4 | 8.43 | Buragohain et al. (2021) |
| Cattle dung | 35 | 6 | 2 | 22.70 | Yadav et al. (2017) |
| Waste activated sludge (WAS) | 23 | 2 | 2 | 13.62 | Gaur et al. (2017) |
| Switchgrass | 48 | 5 | 1 | 36.21 | Buragohain et al. (2021) |
| Rice straw | 39 | 4 | 1 | 70.00 | Buragohain et al. (2021) |

### 1.4. Nutrient Recovery

Duckweed is an ideal biomass for biogas production via anaerobic digestion because it is decoupled from land use and can simultaneously recover nutrients while providing biomass for bioenergy production. Duckweed has been discussed and utilized as a tertiary wastewater treatment for municipal, industrial, and agricultural wastewater for decades (Cheng and Stomp, 2009). Engineers have used duckweed plants in hybrid wetland designs to remove contaminants from anaerobic sludge (Tufaner, 2020). The fast-growing aquatic plant has also been recognized as an alternative to the common and concerning practice of spray-field crop irrigation by recovering nutrients while growing in wastewater. Cheng and Stomp (2009) evaluated the nutrient recovery capabilities of duckweeds that displayed high growth rates, nitrogen (N) and phosphorus (P) removal rates, and tolerance to artificial and anaerobically-treated swine wastewater (Cheng and Stomp, 2009). The presence of a nutrient reserve within the plant and its relationship to biomass growth was observed during the analysis of nutrient removal by the plant. Surprisingly, duckweed continued to grow at similar doubling times after it had depleted N and P in the wastewater and when it was completely removed from the nutrient source (Cheng and Stomp, 2009). This demonstrated the resilience and stability of the prospective energy crop as well as the breadth of the nutrient reserve it can build. Toyama et al. (2018) measured nitrogen removal and biomass growth of four duckweed varieties: *Spirodela polyrhiza*, *Lemna minor*, *Lemna gibba*, and *Landoltia punctata* grown in municipal wastewater, swine wastewater, and anaerobic effluent from a human fecal sludge treatment system. *S. polyrhiza* was the best variety in terms of nitrogen removal from all effluents and also had the highest growth rate (Toyama et al., 2018). This impressive ability to remove N from wastewaters has made duckweed a candidate for phytoremediation.

Many water bodies are in dire need of environmental remediation technology as consequences of fertilizer and pesticide use become more severe (Liu et al., 2021). One consequence of water pollution is eutrophication. Liu et al. (2021) conducted a review focused on the use of duckweed for water pollution remediation and subsequent bioenergy production

(Liu et al., 2021). Duckweed displayed the capacity to restore eutrophic water as well as industrial, municipal, and agricultural wastewaters with its nutrient uptake capabilities. By cultivating duckweed in contaminated water, the plant was provided necessary concentrations of nitrogen and phosphate. Maximum N and P removal rates of 98.0% and 98.8% were calculated from field-testing lagoons (Liu et al., 2021). However, it is important to consider bacteria and microalgae that likely grew in symbiosis with the duckweed biomass within these field lagoons. The compatibility of duckweed and nitrogen-fixing bacteria has inspired studies with bacterium intentionally introduced to duckweed aquatic phytoremediation systems. One of such studies recognized increases in biomass yield, chlorophyll content, stability, and removal efficiency of the system (Liu et al., 2021).

North Carolina has an abundance of swine waste lagoons that offer potential for duckweed production with simultaneous nutrient sequestration (Bergmann et al., 2000a). It is consistent then that many studies of duckweed's nutrient sequestration capacity conducted in North Carolina have utilized swine wastewater as the nutrient-rich substrate for the plant. Cheng et al. (2002) studied the nutrient removal abilities of *Lemna minor 8627* grown in swine wastewater and made several notable observations (Cheng et al., 2002). Duckweed growth and nutrient removal rates increased in response to greater effluent dilutions, and at lower nutrient concentrations, assimilation was the dominant mechanism for N and P removal (Cheng et al., 2002). Cheng et al. (2002) studied the nutrient uptake trends of *Spirodela punctata 7776* grown in swine wastewater and recognized that N and P utilization was linear with duckweed growth (Cheng et al., 2002). Table 1.5. details optimized nutrient uptake rates of reviewed studies.

**Table 1.5.** Compilation of reviewed nutrient (nitrogen and phosphorus) uptake rates from duckweed grown in swine wastewater.

| **Duckweed Species** | **Nutrient Uptake Rate** | | **Reference Literature** |
|---|---|---|---|
| | **Nitrogen** | **Phosphorous** | |
| *Lemna minor* | 0.140 g m$^{-2}$ day$^{-1}$ | 0.004 g m$^{-2}$ day$^{-1}$ | Pena et al. (2017) |
| *Spirodela punctata 7776, Lemna minor 8627, & Lemna gibba 8678* | 1.3 g m$^{-2}$ day$^{-1}$ | 0.18 g m$^{-2}$ day$^{-1}$ | Cheng and Stomp (2009) |
| *Lemna P1* | 0.622 g m$^{-2}$ day$^{-1}$ | 0.135 g m$^{-2}$ day$^{-1}$ | Xiao et al. (2013) |
| *Spirodela punctata 7776* | 0.955 g m$^{-2}$ day$^{-1}$ | 0.129 g m$^{-2}$ day$^{-1}$ | Cheng et al. (2002) |
| *Lemna minor 8627* (in vitro) | 3.36 g m$^{-2}$ day$^{-1}$ | 0.20 g m$^{-2}$ day$^{-1}$ | Cheng et al. (2002) |
| *Lemna minor 8627* (field) | 2.11 g m$^{-2}$ day$^{-1}$ | 0.59 g m$^{-2}$ day$^{-1}$ | Cheng et al. (2002) |

### 1.5. Biogas Production

Anaerobic digestion of sustainably sourced biomass to generate biogas is a vital form of renewable energy that provides significant benefits to the environment. This is especially true when the feedstock for this bioenergy production does not threaten food or feed security. Production of bioenergy from duckweed biomass has been studied for decades with a focus on bioethanol production via fermentation and biogas production via anaerobic digestion (J. J. Cheng and Stomp, 2009; Nahar and Sunny, 2020). Calicioglu and Brennan (2018) evaluated bioenergy yields from sequential fermentation and digestion of duckweed biomass (Calicioglu and Brennan, 2018). Upstream ethanol fermentation prior to anaerobic digestion of duckweed resulted in greater bioenergy yields. However, it was noted that anaerobic digestion of duckweed was more feasible than large-scale ethanol fermentation (Calicioglu and Brennan, 2018). Rana et al. (2021) came to a similar conclusion from a sequential bioethanol-biomethane fermentation experiment in that biogas production from duckweed without prior ethanol production resulted in better bioenergy yields.

Duckweed has a high nitrogen concentration that supports a lower C/N ratio than what is ideal for anaerobic digestion (Chen et al., 2022). To combat this problem and increase carbon concentrations in the feedstock, duckweed has often been studied as a feedstock for co-digestion. Pena et al. (2017) conducted an evaluation of nutrient removal from swine wastewater by duckweed followed by co-digestion of the plant with swine manure (Pena et al., 2017). The digesters that processed both duckweed and swine manure showed stable C/N ratios and higher methane production rates than the digesters that processed single feedstocks (Pena et al., 2017). This increase could have been attributed to the increase in microbial diversity within the co-digestion reactors that was supported by the more complex mixed feedstock. Verma and Suthar (2015) presented a review of existing material related to energy production from duckweed biomass (Verma and Suthar, 2015). While the practice of anaerobic digestion of duckweed as a single feedstock was not evaluated, the successful co-digestion of duckweed and chicken manure was referenced (Verma and Suthar, 2015). One point of interest that was not discussed was the ultimate analysis of the poultry manure digested. Poultry manure, like duckweed, has a high concentration of nitrogen which likely wouldn’t increase the C/N ratio. The inclusion of an ultimate analysis would clarify the C/N ratio of the mixed feedstock. Buragohain et al. (2021) conducted evaluations of several co-digestion scenarios, including duckweed co-digested with cow manure (Buragohain et al., 2021). By combining the two feedstocks, the cumulative biogas production from the anaerobic digestion system increased by roughly 50 mL of biogas per gram volatile solids (Buragohain et al., 2021).

A principal advantage of duckweed over conventional lignocellulosic feedstocks for anaerobic digestion is that duckweed does not require intensive pretreatment to successfully produce biogas (Calicioglu and Brennan, 2018). The small size of duckweed enables it to be fed to large-scale anaerobic digesters without mechanical pretreatment (e.g., milling or grinding), which substantially reduces energy inputs of the anaerobic digestion system (Verma and Suthar, 2015). Nevertheless, several studies have still evaluated different pretreatment strategies as methods to improve the anaerobic digestion of duckweed. Rana et al. (2021) utilized chemical pretreatment in their design of a sequential bioethanol and biogas biorefinery concept using Spirodela duckweed that has been nutrient starved to increase carbohydrate concentrations. Prior to bioprocessing the plant, a dilute acid pretreatment was utilized to produce monomeric glucose from plant starch, with a 99.7% conversion rate (Rana, Khan, Shiekh, et al., 2021). This pretreatment choice was supported by a previous study that found the acid treatment to increase glucose accessibility to improve bioethanol production via fermentation (Rana, Khan, Irfan, et al., 2021). This particular pretreatment was not discussed as an improvement strategy for anaerobic digestion of the duckweed biomass, however, the study did conclude that anaerobic digestion, without previous ethanol fermentation, would be the most efficient pathway for energy production from duckweed. Tonon et al. (2017) performed batch digestions of duckweed grown in wastewater to evaluate three pretreatment strategies for notable biogas production improvements. The strategies selected involved drying the duckweed at 35ºC for 24 hours (drying group), combining the duckweed with a 1% NaOH solution for 24 hours (alkaline group), and fermenting the biomass with the anaerobic digestion inoculum for three days (fermentation group) (Tonon et al., 2017). Results from this study showed that all three pretreatment groups produced more methane per gram volatile solids fed when compared to the untreated duckweed biomass. Finally, it was recommended by Tonon et al. that the simplest pretreatments with increased biomethane production for existing systems would be solar drying (drying at 35ºC) and fermentative pretreatment.  Guar et al. conducted thermal pretreatment of duckweed biomass and waste activated sludge before processing the feedstocks in anaerobic digesters for biogas production. This pretreatment involved autoclaving the substrate materials separately at 120 ºC for 30 minutes to increased solubilization of organic matter and therefore reduce inhibition within the hydrolysis process (Gaur et al., 2017). All batch reactors processing thermally pretreated materials produced more methane than their non-pretreated counterparts. While studies evaluating pretreatment strategies have noted increased methane production from the biomass, it has not been specified if the energy required for the pretreatment is smaller than the increase in overall energy produced. Duckweed-based anaerobic digestion, without pretreatment, still produces a notable amount of methane, without requiring extensive energy inputs.

Specific methane productions of duckweed types (with and without pretreatment) and common biogas energy crops are listed in Table 1.6.. In each study that evaluated the addition of duckweed to a common anaerobic digestion feedstock (e.g. municipal wastewater, food waste, animal manure, waste activated sludge) an increase in methane production was observed (Gaur and Suthar, 2017; Navarro et al., 2022; Pena et al., 2017). It is important to note that in each of the referenced experiments, anaerobic digestion (conducted in continuous or batch systems) was operated at mesophilic temperatures.

**Table 1.6.** Compilation of reviewed specific methane production rates from Lemnaceae biomass with and without pretreatment and common anaerobic digestion feedstocks.

| Feedstock | Pretreatment | Specific Methane Production | | Reference Literature |
|---|---|---|---|---|
| | | Value | Unit | |
| Duckweed and municipal wastewater | None | 340.0 | NL $CH_4$ $kg^{-1}$ VS | Toyama at al. (2018) |
| Duckweed and swine wastewater | | 361.0 | | |
| Duckweed and anaerobic digestate | | 413.0 | | |
| Duckweed | Dried | 190.0 | NL $CH_4$ $kg^{-1}$ VS | Tonon et al. (2017) |
| | Alkaline | 190.0 | | |
| | Fermentation | 230.0 | | |
| Food waste | None | 232.8 | NL $CH_4$ $kg^{-1}$ VS | Navarro et al. (2022) |
| Food waste and Duckweed | Blended and dried | 369.7 | | |
| Duckweed and Swine Wastewater | None | 131.0 | NL $CH_4$ $kg^{-1}$ COD | Pena et al. (2017) |
| Swine Wastewater | | 93.0 | | |
| Swine Manure | None | 443.6 | NL $CH_4$ $kg^{-1}$ VS | Cu et al. (2015) |
| Cow Manure | | 222.1 | | |
| Duckweed | | 340.6 | | |
| Water Spinach | | 110.6 | | |
| Duckweed and Waste Activated Sludge | Thermal | 468.0 | NL $CH_4$ $kg^{-1}$ VS | Gaur et al. (2017) |
| Duckweed and Waste Activated Sludge | None | 76.0 | | |
| Duckweed and Waste Activated Sludge (5:1) | Blended | 168.1 | NL $CH_4$ $kg^{-1}$ VS | Gaur and Suthar (2017) |
| Duckweed and Waste Activated Sludge (2:1) | | 164.0 | | |
| Duckweed and Canned Seafood Wastewater | None | 352.0 | NL $CH_4$ $kg^{-1}$ COD | Panpong et al., (2014) |
| Canned Seafood Wastewater | | 115.0 | | |
| Sugar Beet | None | 374.9 | NL $CH_4$ $kg^{-1}$ organic dry matter | Herrmann et al., (2016) |
| Maize | | 328.2 | | |
| Miscanthus | | 217.2 | | |
| Potato | | 330.6 | | |

## 1.6. Future Perspectives

Future studies regarding the use of duckweed for biogas production via anaerobic digestion should be concerned with several existing knowledge gaps in the scientific literature. A common theme within studies regarding anaerobic digestion of duckweed is the call for technoeconomic analyses of the biomass and related energy production. Storage, collection, and transportation solutions are still needed for this biomass to be a viable feedstock for biogas production on a larger scale (Verma and Suthar, 2015). The completion of life cycle assessments considering the environmental and bioeconomic implications would enable this bioenergy process to have a broader impact. This impact would also be complimented by deeper investigations into bioenergy produced by duckweed biomass.

While previous research has been productive, knowledge gaps still exist regarding the potential for thermophilic anaerobic digestion or co-digestion of duckweed. Many studies have focused on mesophilic temperatures for reasons including the lower energy required to maintain temperature and the increased stability at mesophilic operating conditions. However, digester stability is not entirely dependent on operating temperature and depends also on substrate composition. While there are potential stability and sensitivity concerns, thermophilic anaerobic digestion enables the operator to conduct continuous digestion at a shorter hydraulic retention time than ideal for mesophilic conditions (Labatut et al., 2014). A shorter HRT is actually better for thermophilic anaerobic digestion because it prohibits long-chain fatty acid accumulation. At shorter HRTs, chemical and biological reactions within the system are happening at an accelerated rate, and the digester is capable of processing more material, faster. Effluent from thermophilic anaerobic digesters, unlike that from mesophilic, can be categorized as a Class A fertilizer because the high temperature bioprocess can remove pathogens that could be contained within the substrate (Insam et al., 2015).

Few studies have been found discussing the idea of thermophilically digesting duckweed biomass. Matosevic et al. conducted batch thermophilic anaerobic digestion experiments using duckweed biomass that had been grown on varying concentration of digestate, and found that as digestate concentration increased past 4.3% there was a decrease in biogas production (Matošević et al., 2019). The choice of thermophilic temperature, as opposed to mesophilic, was not discussed. Calicioglu et al. evaluated the effect that temperature and pH have on anaerobic digestion of duckweed using a batch reactor design (Ozgul Calicioglu et al., 2018). While methane production in this study was much lower than other reported values, they determined that the ideal performance (based on duckweed to carboxylic acid conversion) was achieved at mesophilic and basic conditions. It was also stated that hydrogen recovery was observed at thermophilic and acidic conditions. Ramaraj and Unpaprom performed batch tests evaluating mesophilic, thermophilic, and room temperature conditions of duckweed-based anaerobic digestion and concluded that mesophilic conditions produced slightly higher biogas and methane (Ramaraj and Unpaprom, 2016). A specific methane production, in terms of volume methane produced per unit mass of substrate/COD/VS was not calculated, and this makes it difficult to compare this study’s findings to other anaerobic digestion systems. This study is cited in recent literature as the reason for operating at mesophilic conditions (Tonon et al., 2017). To the knowledge of this literature review, a continuous thermophilic system has not been developed using duckweed coupled with a second feedstock to support better anaerobic digestion conditions.

### 1.7. Conclusion

As industries continue to deplete nonrenewable resources, such as fossil fuels, for energy, researchers are quickly searching for replacement energy sources. A massive shift from environmentally damaging fossil fuels is needed not only because we are exhausting nonrenewable resources, but because we cannot afford the environmental consequences of continued usage. Biofuels have the capabilities to play a major role in this shift, and the field of biofuel production is constantly innovating. One popular biofuel is biogas, and a relatively simple mechanism for producing biogas is anaerobic digestion (Cheng and Stomp, 2009). Anaerobic digestion of sustainably sourced biomass to generate biogas is a vital form of renewable energy that provides significant benefits to the environment. Current biogas production from conventional feedstocks (e.g., agricultural wastes and residues) is inadequate when considering our growing energy demands. Alternatively, the use of sustainably grown energy crops that do not occupy arable land has attracted attention for biogas production. An ideal example of such biomass is the aquatic plant lemnaceae, commonly known as duckweed.

Duckweed is a fast-growing aquatic plant with great potential for biogas production via anaerobic digestion. Duckweed is one of the fastest growing plants on earth and can be continuously harvested and anaerobically digested with no required pretreatment (Liu et al., 2021). The carbohydrate composition of duckweed enables simple hydrolysis during digestion seeing that the plant is very low in lignin (Yadav et al., 2017). The chemical composition of duckweed is higher in nitrogen than other conventional anaerobic digestion feedstocks (Pena et al., 2017). Therefore, duckweed-based biogas production is optimized via co-digestion with carbon-heavy biomass such as agricultural wastes or residues. Duckweed is a nutrient dense feedstock because it proficiently takes nutrients from the water on which it grows. This attribute has enabled duckweed to be used as an intermediate or tertiary wastewater treatment technique for agricultural, industrial and municipal wastewaters. Past studies evaluating the biogas production from duckweed have shown success with mesophilic digestion. However, there are still gaps in the scientific literature regarding duckweed-based biogas production. These gaps include the potential to continuously anaerobically digest and co-digest duckweed at thermophilic temperatures. Review of existing scientific literature shows that duckweed is an ideal candidate for biogas production via anaerobic digestion.

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# CHAPTER 2: CONTINUOUS ANAEROBIC CO-DIGESTION OF LEMNACEAE AND SWINE WASTEWATER FOR BIOGAS PRODUCTION

## 2.1. Introduction

Bioprocesses that produce renewable energy effectively without requiring intensive energy inputs are essential to replace quickly depleting fossil fuel resources. One of these processes is anaerobic digestion for the production of biogas, a combination of methane and carbon dioxide that, after purification, can serve as a direct replacement to natural gas (Rouches et al., 2016). Anaerobic digestion can also produce nutrient-dense fertilizer, remove pathogens from substrates, and reduce greenhouse gas emissions (AgSTAR, 2020). Common feedstocks for anaerobic digestion include agricultural and municipal wastes and many of these systems have been implemented at industrial scales (Mutungwazi et al., 2021). However, biogas production from waste materials is insufficient when compared to the tremendous need for renewable energy. Energy crops, grown for the sole purpose of biogas production, have been well-researched and implemented to increase methane production within anaerobic digestion systems. However, most energy crops are harvested only a few times a year, thus requiring storage programs, and must be grown on arable land that would otherwise be used to grow food or animal feed (Hashemi et al., 2021). Alternatively, an aquatic plant that can be grown and harvested continuously within waste or contaminated water could provide a stable substrate for anaerobic digestion (Markou et al., 2018). One such plant is Lemnaceae, commonly referred to as duckweed, and has already shown great potential for biogas production (Yadav et al., 2017).

Duckweed is a small free-floating plant that has a wide geographical distribution and an incredibly fast growth rate with a doubling time of 16 – 24 hours under ideal conditions (Cheng and Stomp, 2009; Peng et al., 2007). Previous research on duckweed-based phytoremediation strategies have found that the plant has the potential to reduce nutrient loading within contaminated water as well as agricultural, industrial, and municipal wastewaters (Nahar and Sunny, 2020). This characteristic has inspired joint wastewater treatment and bioenergy systems in which duckweed is grown in a wastewater substrate and then used as a feedstock for bioethanol, biohydrogen, or biogas production (Calicioglu et al., 2021; Toyama et al., 2018; Xu and Deshusses, 2015). However, evaluation of separate and joint duckweed-based bioethanol and biomethane production systems have concluded that direct biomethane production, via anaerobic digestion, is the most viable form of energy recovery from the biomass (Calicioglu and Brennan, 2018; Calicioglu et al., 2021).

Duckweed is an ideal feedstock for anaerobic digestion because it can be grown and harvested continuously, enabling constant energy production with little storage requirements, and because it lacks the recalcitrant cell wall structure that often inhibits successful hydrolysis (Cui and Cheng, 2015; Tonon et al., 2017). However, high nitrogen contents of many duckweed types often contribute to a lower C/N ratio than what is ideal for single-substrate anaerobic digestion, and a second feedstock is necessary to increase carbon contents (Chen et al., 2022). Cow manure, swine wastewater, canned seafood wastewater, waste activated sludge, and foodwaste substrates have been combined with duckweed and have resulted in increased methane production rates (Gaur et al., 2017; Gaur and Suthar, 2017; Navarro et al., 2022; Panpong et al., 2014; Pena et al., 2017; Toyama et al., 2018). All of these studies conducted anaerobic digestion at mesophilic conditions, likely due to the increased stability and resiliency of mesophilic microbiomes (Labatut et al., 2014). Few studies that have previously evaluated duckweed-based anaerobic digestion at thermophilic conditions, but all have done so through the use of batch digesters (Calicioglu et al., 2018; Matošević et al., 2019; Ramaraj and Unpaprom, 2016).

The objective of this study was to determine if duckweed biomass is a viable candidate for continuous thermophilic anaerobic co-digestion with swine wastewater. A second goal is to determine key digester performance parameters, such as specific methane production ($m^3$ $CH_4$ $kg^{-1}$ $COD_{consumed}$), that will enable the design of an optimized thermophilic anaerobic digestion system. The experiment required the establishment of a stable continuous thermophilic anaerobic digestion system processing swine wastewater and duckweed at four different organic loading rates.

## 2.2. Materials and Methods

### 2.2.1. Inoculum Preparation

The inoculum used to initially stabilize the digesters was culture taken from operational mesophilic anaerobic digesters processing municipal waste solids at the South Durham Water Reclamation Facility (SDWRF). The inoculum was taken from the SDWRF and immediately placed within the Continuously Stirred Tank Reactor (CSTR) tanks at a temperature of 35ºC. The CSTR tanks were filled to 5 and 7.5 L working volumes with the mesophilic culture.

### 2.2.2. Swine Wastewater

Swine wastewater was used as the feedstock for the control period of the experiment and used as a co-feedstock with the duckweed biomass. Swine wastewater was collected from the settling pond at the Swine Education Unit of the Lake Wheeler Road Field Laboratory of North Carolina State University (Raleigh, NC) and stored at 4 ºC. Wastewater characteristics are shown in Table 2.1. New batches of swine wastewater were immediately analyzed for chemical oxygen demand (COD) to ensure consistent concentrations throughout the experiment using the HACH reactor digestion method (Method 8000, HACH Co., Loveland, CO.).

**Table 2.1.** Swine wastewater characteristics.

| **Parameter** | **Swine Wastewater** |
|---|---|
| Total Kjeldahl Nitrogen (mg/L) | 270.0 |
| Chemical Oxygen Demand (mg/L) | 6238 |
| Total Phosphorus (mg/L) | 120.0 |
| Total Solids (%) | 0.28 |
| Volatile Solids (%) | 52.06 |

### 2.2.3. Lemnaceae Preparation

The Lemnaceae plants used in this study were grown and harvested at the Lake Wheeler Road Field Laboratory of North Carolina State University (Raleigh, NC) adjacent to the swine wastewater lagoon. The duckweed was grown in a 300 sq ft hydroponic system utilizing swine wastewater as a fertilizer and harvested twice per week. The harvested biomass was put into mesh bags and allowed to air dry for several days, then it was spread out to dry in the sun before being stored at a moisture content of 10-15%. Two of the three varieties chosen for this study were selected based on findings from previous research studying the plant's ability to sequester nutrients from swine wastewater (Bergmann et al., 2000b, 2000a). Accession numbers for these two varieties from the Rutgers Collection (RDSC, http://www.ruduckweed.org/) are Lg8678 and Lg7741 for the *Lemna Gibba* (Raleigh) and *Lemna Gibba* (Sicily), respectively. The third and final duckweed variety was found growing in a pond approximately 50 miles from the NC State University campus and was collected by the project's research technician Michael Adcock. This variety has not yet been genotyped at this time but it is theorized that it is of the family *Spirodela* and has the shorthand name Culbreth. The Lemna varieties obtained for this study will be referenced to by their shorthand names: Raleigh, Sicily, and Culbreth.

Dried duckweed biomass was collected and brought back to Weaver Laboratories at North Carolina State University (Raleigh, NC) where all subsequent experiments took place. Previous CSTR trials using whole dried duckweed biomass proved that mixing would be difficult as the plant continued to float within the digesters. In order to maintain consistent mixing within the reactors, the biomass was mechanically pretreated. The biomass was milled using a biomass mill (IKA MF10) with a 0.25 sieve (0.25 mm hole size) and stored in plastic bags at room temperature. Biomass Characteristics are shown in Table 2.2..

**Table 2.2.** Lemnaceae biomass characteristics.

| Parameter | Sicily | Culbreth | Raleigh |
|---|---|---|---|
| Total Kjeldahl Nitrogen (mg/L) | 43200 | 47720 | 44240 |
| Chemical Oxygen Demand (mg/kg) | 898500 | 913700 | 916100 |
| Total Phosphorus (mg/L) | 26470 | 14740 | 23470 |
| Total Solids (%) | 88.52 | 96.79 | 89.53 |
| Volatile Solids (%) | 74.84 | 84.39 | 75.89 |

### 2.2.4. Continuously Stirred Tank Reactor (CSTR) Operation

Three CSTR digesters were used to conduct continuous anaerobic digestion for this experiment: two 7 L with 5 L working volumes (Applikon Bioconsole ADI 1035, Rochester, NY)  and one 11 L with 7.5 L working volume (BioFlo 110, New Brunswick, NJ). The reactors herein are referred to as Lapp (Left-Applikon), Rapp (Right-Applikon) and BioFlo. The three digesters were inoculated with anaerobic sludge at mesophilic temperatures (35 ºC) and transitioned to thermophilic conditions according to a method outlined by Shin et al. (2019), by raising the temperature 5ºC each day for 3 days while the digesters were starved. During the experiment, digester contents were agitated at constant rates of 200 rpm and maintained at 50 ºC by heating tape (Applikon configurations) and a recirculating water jacket (BioFlo). The hydraulic retention time (HRT) for each digester was kept constant at 10 days throughout the entire experiment. The loading rates with COD and TS concentrations for the four phases of the experiment are listed in Table 2.3. COD was chosen as the metric for organic loading rate because it is an accurate measure of organics in the substrate (Meegoda et al., 2018). The first phase of the digestion served as a baseline, or control, in which the digesters were fed swine wastewater and ethanol to supplement the COD concentration, this was conducted for 45 days to ensure digester stability before the addition of duckweed biomass. The second, third, and fourth loading rates incorporated dried duckweed biomass in increasing amounts with the same amount of swine wastewater and ethanol. These phases were conducted for a minimum of 25 days that accounted for time required for the digesters to stabilize under the new loading rate. The entire experiment was conducted over 125 days beginning on August 20, 2022, and concluding on December 23, 2022. Each digester was fed one duckweed type for the entire experiment with the Lapp processing Culbreth, Rapp processing Raleigh, and BioFlo processing Sicily duckweeds. Biogas production and pH were measured daily, and this raw data is recorded in Table A-1.

**Table 2.3:** Continuous digestion system organic loading rates. Organic loading rate in percent total solids (in substrate) and mg COD/L digester/day for each phase of the experiment.

| **Loading Rate** | **Total Solids in Feed (%)** | **COD (mg/L digester/day)** |
|---|---|---|
| Control | 0.3 | 1,000 |
| Organic Loading Rate 1 | 1.3 | 2,000 |
| Organic Loading Rate 2 | 2.3 | 3,000 |
| Organic Loading Rate 3 | 4.3 | 4,500 |

Biogas production was measured using wet tip gas meters (WetTipGasMeter.com, Nashville, TN) where the biogas was continuously monitored by electromagnetic sensors reporting tip counts that were recorded and reset daily. Tip meters were recalibrated every 20 days by repeatedly injecting known quantities of air into the meter to measure the necessary volume to account for a single tip.

### 2.2.5. Analytical Methods

pH was measured daily using an accuTupH™ pH electrode (Fisherbrand™, Waltham, MA). Samples were collected weekly and sent to the Environmental Analysis Lab at North

Carolina State University for the measurements of total solids (TS), volatile solids (VS), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), and total phosphorous (TP) according to APHA Standard Methods (APHA, 1998). Biogas composition was measured weekly using a Shimadzu gas chromatograph (GC) (Shimadzu GC-2014, Kyoto, Japan) with a thermal conductivity detector and equipped with a 3 m by 3 mm 100/120 Carbosieve SII packed column (Supelco, Bellefonte, Pennsylvania) with helium as the carrier gas.

### 2.2.6. Data Analysis

Digester differences were evaluated using analyses of variance (ANOVA) with significance of $p < 0.05$. All data analysis was performed with Excel and R Studio

## 2.3. Results and Discussion

### 2.3.1. Biogas Production

Figure 2.1. shows the performance of the three CSTR digesters regarding daily biogas produced throughout the experiment, normalized to mL biogas per L digester. The plot shows a trend: increasing organic loading rates increased biogas production significantly ($p < 0.05$). This is supported by the literature and the theory that increasing organic loading rate can increase biogas production up to a threshold at which the system may become overloaded (Kundu et al., 2017). The continued increase in biogas production throughout this experiment implies that this threshold was not met. The highest average daily biogas volumes produced were 1554, 1531, and 1540 mL biogas per liter digester working volume from the Lapp, Rapp, and BioFlo digesters, respectively, during OLR3. This corresponds to biogas production yields of 0.345, 0.340, and 0.342 $m^3$ biogas $kg^{-1}$ $COD_{consumed}$. Comparatively, this result is similar to the yield produced by Weidong et al. (2013) who reported a biogas yield of 0.31 L biogas $g^{-1}$ $COD_{consumed}$ from anaerobic digestion of duckweed biomass and swine manure. This previous study was conducted

at mesophilic temperatures and processed swine manure and duckweed biomass at a 1:1 ratio with a retention time of 35 days (Weidong et al., 2013). In terms of biogas yield, the thermophilic system developed in this study performed as well as its mesophilic counterpart, and with a much shorter HRT. Shorter HRTs translate to higher loading rates, increased efficiency, and lower capital costs (Meegoda et al., 2018).

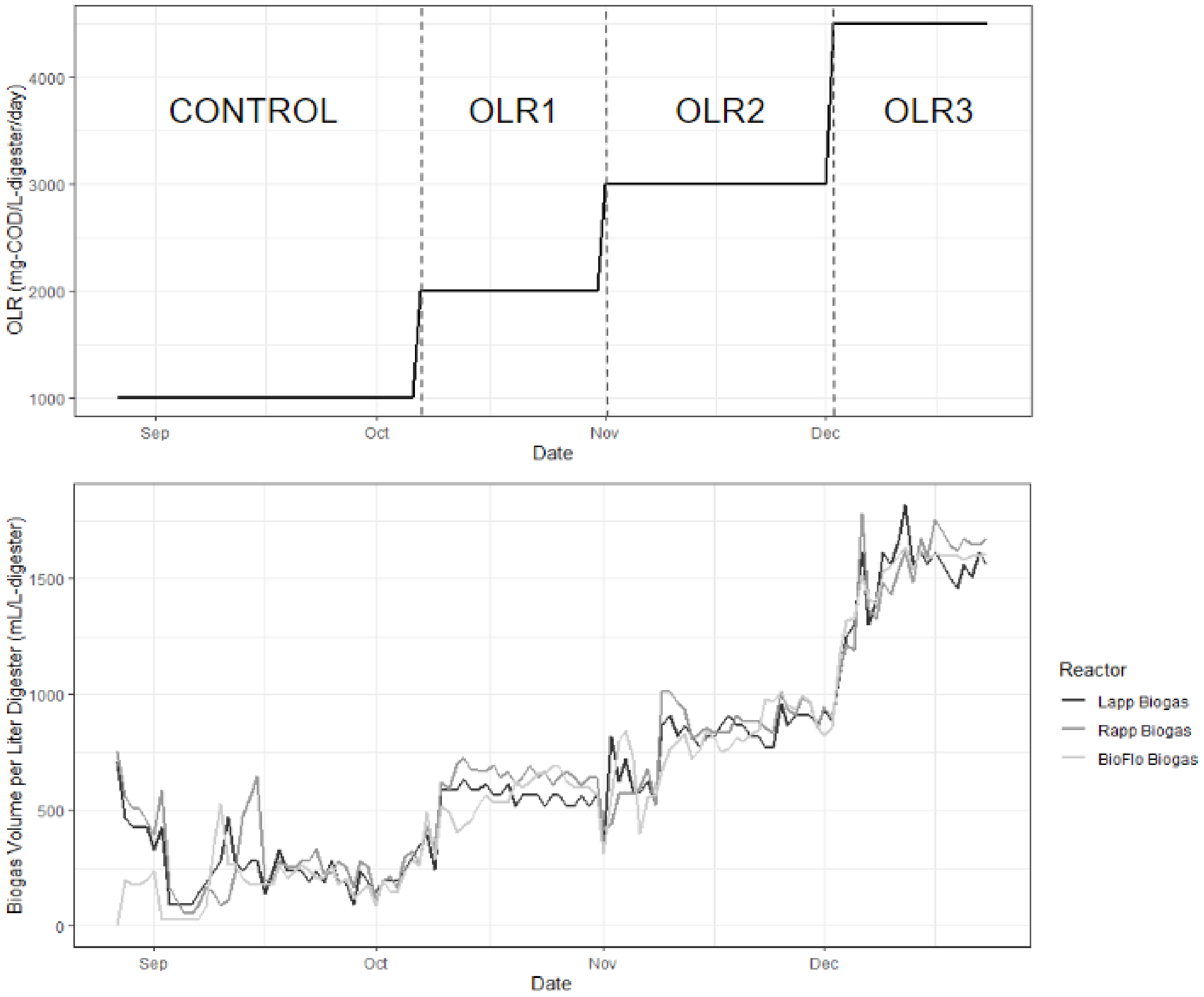

**Figure 2.1.** Daily biogas production volume. Daily biogas production volumes from three CSTR normalized per volume anaerobic digester (bottom) throughout the four phases of the experiment (top).

### 2.3.2. Methane Production

Methane concentrations (by percent volume) evaluated via gas chromatography analysis throughout the four phases of the experiment are summarized in Table 2.4. During the control phase, the three digesters did not produce significantly different ($p > 0.5$) concentrations of methane within the produced biogas. The average methane concentration across the three digesters during the control phase was 68.41% ± 1.76%. The methane contents of the biogas while duckweed was being processed ranged from 53.76% to 61.55% and were not significantly different ($p > 0.05$) between CSTRs or loading rates at each OLR with the exception of OLR1 (likely due to the initial adjustment from processing a single to a mixed substrate). The methane concentrations observed in this experiment were similar to those produced by Matošević et al. (2019) who conducted thermophilic digestion of duckweed biomass grown in a digestate medium and reported a methane content of 56%.

The control phase produced significantly ($p < 0.05$) higher methane concentrations by percent volume than the other organic loading rates and the methane concentrations of the biogas decreased following the addition of duckweed biomass and continued to decrease as the mass of duckweed increased. This trend is supported by the idea that the introduction of more complex polymers (e.g. cellulose, hemicellulose, lignin) can cause inhibition of methanogens and increased volatile fatty acid (VFA) accumulation (Li et al., 2018). VFA accumulation is often used as a metric of stability and would be a worthy avenue of future research for duckweed-based thermophilic anaerobic digestion. Additionally, the decrease in methane (by percent volume) can be attributed to the increase in nitrogen within the digester upon the addition of duckweed, which is largely composed of protein. The decrease in methane (by percent volume) observed in this study is opposite of that reported by Pena et al. (2017) in a similar study. This previous experiment operated CSTRs with a 10-day HRT processing swine wastewater with and without duckweed biomass at mesophilic conditions. The methane concentrations observed in this previous study increased upon the addition of duckweed from approximately 50% to 60% methane while the OLR increased from 0.83 g COD $L^{-1}$ $day^{-1}$ (swine wastewater only) to 0.99 g COD $L^{-1}$ $day^{-1}$ (swine wastewater and duckweed) (Pena et al., 2017). While at the surface level this represents an opposing trend to this study, the OLRs investigated were much lower than those chosen for this experiment and the absence of experimental data taken with comparable organic loading rates minimizes one's ability to directly compare the two studies. The increase in methane concentration observed by Pena et al. could be attributed to the organic loading rate nearing the optimal range of 1.2 – 9.0 g COD $L^{-1}$ $day^{-1}$ (Rincón et al., 2006).

**Table 2.4.** Biogas methane concentrations throughout continuous digestion experiment. Average methane concentrations from biogas produced from three CSTRs throughout four phases of experiment.

| **Loading Rate** | **Methane Concentration (% vol.)** | | |
|---|---|---|---|
| | **Lapp** | **Rapp** | **BioFlo** |
| Control | 66.49 ± 0.32 | 68.77 ± 0.57 | 69.96 ± 0.29 |
| Organic Loading Rate 1 | 61.55 ± 1.73 | 56.28 ± 0.68 | 59.09 ± 0.52 |
| Organic Loading Rate 2 | 57.44 ± 0.94 | 56.69 ± 0.31 | 56.42 ± 1.98 |
| Organic Loading Rate 3 | 55.00 ± 2.00 | 55.98 ± 1.21 | 53.76 ± 2.00 |

Daily methane volumes produced per liter digester throughout the experiment are displayed in Figure 2.2. The highest average methane volumes produced by each digester were 941, 987, and 921 mL $CH_4$ per L digester working volume for the Lapp, Rapp, and BioFlo reactors, respectively, and were produced during OLR3. While the concentration of methane within the biogas decreased, the biogas production increased with the increasing OLR and resulted in higher volumes of methane produced.

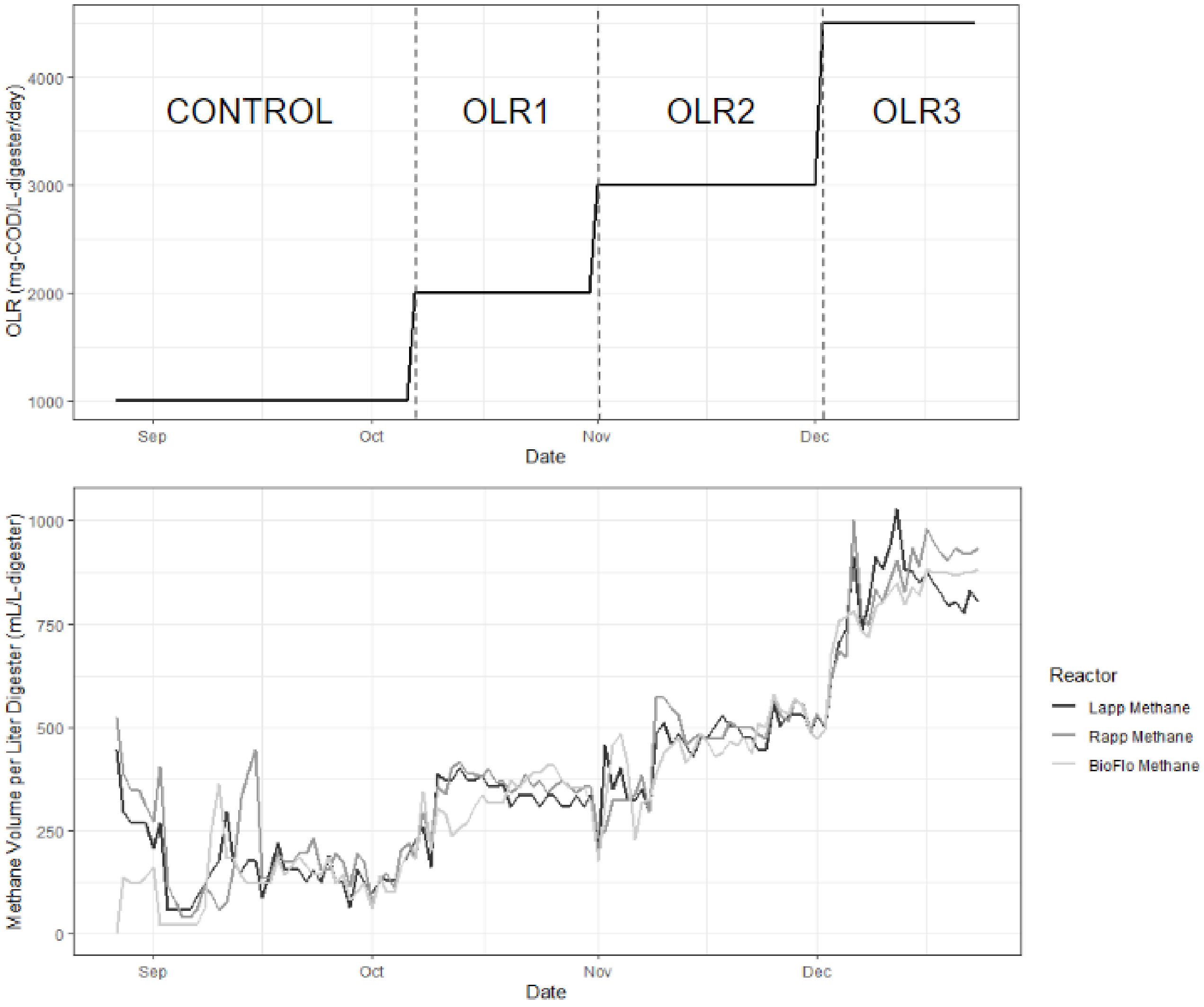

**Figure 2.2.** Daily Methane Production Volume. Daily methane production volumes from three CSTR normalized per volume anaerobic digester.

### 2.3.3. CSTR Performance

Table 2.5 shows the COD reduction rates for each digester during the four phases of the experiment. The highest COD reduction efficiencies were 71%, 72%, and 70% for the Lapp, Rapp, and BioFlo digesters, respectively, and were attained during the control phase when the digesters were fed the lowest organic loading rate. COD reduction rates were never significantly different ($p > 0.05$) between the three digesters at the same OLR, showing no effect of duckweed type on COD reduction rate. There was significant difference in COD reduction between sequential OLR steps. This trend is expected from literature review: reduction efficiency decreases as the COD loading rate increases (Musa et al., 2018; Panpong et al., 2014; Paulo et al., 2020; Waqas et al., 2021). In anaerobic digestion systems, COD is a measurement of organics present in the influent and effluent, and COD reduction or removal reflects the degradation rates within the digester (Meegoda et al., 2018). As OLR increases, more COD (mg $L^{-1}$) is being removed by the system but at a lower rate because the substrate is putting greater loading on the microbial community.

**Table 2.5.** COD reduction rate for each phase of the experiment.

| **Loading Rate** | **COD Reduction Rate (%)** | | |
|---|---|---|---|
| | **Lapp** | **Rapp** | **BioFlo** |
| Control | 71.0 ± 0.8 | 71.6 ± 1.3 | 70.2 ± 1.2 |
| Organic Loading Rate 1 | 67.1 ± 10.0 | 66.3 ± 7.5 | 68.5 ± 3.7 |
| Organic Loading Rate 2 | 57.4 ± 2.5 | 58.2 ± 3.4 | 58.7 ± 2.9 |
| Organic Loading Rate 3 | 53.1 ± 3.2 | 56.1 ± 2.3 | 56.5 ± 2.7 |

The COD conversion rate determined by Weidong et al. (2013) during co-digestion of duckweed biomass and swine manure was 63.2%, which falls within the range (53.1 – 68.5%) described in this study. COD removal measured by Pena et al. (2017) in their duckweed and swine wastewater anaerobic co-digestion study was 58.9 ± 2%, which is consistent with this study's findings.

Table 2.6 lists the specific methane production rates ($m^3$ $CH_4$ $kg^{-1}$ $COD_{consumed}$) for each digester at each phase of the experiment. While it was observed in Table 2.5 that the COD removal rate decreased across the entire experiment, the methane production rates all increased as the OLR increased. This can be supported by the markedly higher daily methane production volumes observed in Figure 2.2. While the COD reduction rate was decreasing, the overall COD loading (mg COD $L^{-1}$ $day^{-1}$) was much higher, as was the methane volume produced daily, which resulted in increasing methane yields per unit mass COD consumed. Methane production increased significantly ($p < 0.05$) for each CSTR and duckweed group as more duckweed was processed. This trend is supported by the fact that higher OLRs, to a point, support higher biogas production rates, and therefore higher methane yields (Rincón et al., 2006). This is true up until an OLR at which the digester performance begins to worsen as a result of process failure. Differences in specific methane production rates between the three digesters were significant ($p<0.05$) at each loading rate except for the control phase (Table 2.7). During the control phase, the three CSTR digesters were fed identical substrates, so this result confirms no differences between the reactors. The significant differences ($p < 0.05$) between the reactors after the addition of duckweed biomass signify differences between the duckweed types.

When the digesters were fed swine wastewater, the average specific methane production rate was 0.207 $m^3$ $CH_4$ $kg^{-1}$ $COD_{consumed}$. Riaño et al. (2011) conducted mesophilic anaerobic digestion of swine wastewater for biogas production and determined a methane production rate of 256 mL $CH_4$ $g^{-1}$ $COD_{consumed}$, or 0.256 $m^3$ $CH_4$ $kg^{-1}$ $COD_{consumed}$, respectively. Pena et al. (2017) also operated mesophilic anaerobic digestion of swine wastewater (as an experimental control to a swine wastewater and duckweed co-digestion system) and found the methane production rate when feeding only swine wastewater to be 0.093 $m^3$ $CH_4$ $kg^{-1}$ $COD_{consumed}$. The result determined by this study falls within the range produced by previous literature for the specific methane production rate for swine wastewater based anaerobic digestion.

The highest methane yields were 0.362, 0.338, 0.317 $m^3$ $CH_4$ $kg^{-1}$ $COD_{consumed}$ for the Culbreth, Raleigh, and Sicily duckweed types, respectively, and were all produced during OLR3. These results are much larger than the result reported from a similar study conducted at mesophilic temperatures by Pena et al. (2017), which was 131 mL $CH_4$ $g^{-1}$ $COD_{consumed}$, or .131

$m^3$ $CH_4$ $kg^{-1}$ $COD_{consumed}$, respectively. This difference can be attributed to the much lower OLR (approximately 1 g COD $L^{-1}$ $day^{-1}$) in the Pena et al. study. The result of this study is similar to that determined by Panpong et al. (2014) from a combined canned seafood wastewater and duckweed digestion system, which was 0.352 $m^3$ $CH_4$ $kg^{-1}$ $COD_{removed}$. Methane production rates, in terms of $COD_{fed}$ were calculated for future comparison with batch tests and are listed in Table 2.8.

**Table 2.6.** Methane yields throughout continuous experiment. Specific methane production rates in $m^3$ per kg $COD_{consumed}$ for each phase of continuous digestion.

| Loading Rate | Lapp | Rapp | BioFlo |
|---|---|---|---|
| Control | 0.199 ± 0.050 | 0.228 ± 0.061 | 0.195 ± 0.056 |
| Organic Loading Rate 1 | 0.283 ± 0.013 | 0.299 ± 0.029 | 0.263 ± 0.038 |
| Organic Loading Rate 2 | 0.307 ± 0.017 | 0.321 ± 0.024 | 0.288 ± 0.033 |
| Organic Loading Rate 3 | 0.362 ± 0.032 | 0.338 ± 0.020 | 0.317 ± 0.031 |

**Table 2.7.** ANOVA P-Values for comparison within continuous experiment. One way ANOVA test p-values of methane production rates from three CSTRs during four phases of experiment.

| Control | | | |
|---|---|---|---|
| **Reactor** | **Lapp** | **Rapp** | **BioFlo** |

| | | | |
|---|---|---|---|
| **Lapp** | 1.0000 | 0.1023 | 0.8102 |
| **Rapp** | | 1.0000 | 0.0771 |
| **BioFlo** | | | 1.0000 |
| **Organic Loading Rate 1** | | | |
| **Reactor** | **Lapp** | **Rapp** | **BioFlo** |
| **Lapp** | 1.0000 | 0.0027 | 0.0306 |
| **Rapp** | | 1.0000 | 0.0018 |
| **BioFlo** | | | 1.0000 |
| **Organic Loading Rate 2** | | | |
| **Reactor** | **Lapp** | **Rapp** | **BioFlo** |
| **Lapp** | 1.0000 | 0.0441 | 0.0391 |
| **Rapp** | | 1.0000 | 0.0016 |
| **BioFlo** | | | 1.0000 |
| **Organic Loading Rate 3** | | | |
| **Reactor** | **Lapp** | **Rapp** | **BioFlo** |
| **Lapp** | 1.0000 | 0.0125 | 0.0001 |
| **Rapp** | | 1.0000 | 0.0297 |
| **BioFlo** | | | 1.0000 |

**Table 2.8.** Methane Production Rates Throughout Continuous Experiment. Specific methane production rates in $m^3$ per kg $COD_{fed}$ for each phase of continuous digestion.

| **Loading Rate** | **Lapp** | **Rapp** | **BioFlo** |
|---|---|---|---|
| Control | 0.147 ± 0.039 | 0.169 ± 0.039 | 0.142 ± 0.038 |
| Organic Loading Rate 1 | 0.171 ± 0.014 | 0.170 ± 0.008 | 0.164 ± 0.024 |
| Organic Loading Rate 2 | 0.183 ± 0.012 | 0.184 ± 0.010 | 0.176 ± 0.017 |
| Organic Loading Rate 3 | 0.192 ± 0.014 | 0.190 ± 0.007 | 0.189 ± 0.007 |

## 2.4. Conclusions

The addition of duckweed biomass to the thermophilic anaerobic digestion of swine wastewater increased biogas production and improved methane yield per unit mass COD consumed as well as COD fed. The Culbreth duckweed type produced the highest methane yield of 0.362 $m^3$ $CH_4$ $kg^{-1}$ $COD_{consumed}$ at OLR3, 4,500 mg COD $L^{-1}$ digester $day^{-1}$, which was the highest organic loading rate investigated. This result supports the need for increased OLR evaluation beyond what was performed in this study. Knowledge gaps in the field of thermophilic anaerobic digestion of duckweed biomass still remain, including a study of the process kinetics.

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# CHAPTER 3: BATCH THERMOPHILIC ANAEROBIC DIGESTION OF LEMNACEAE FOR BIOGAS PRODUCTION

## 3.1. Introduction

Lemnaceae, commonly referred to as duckweeds, are some of the fastest growing plants in the world and have been well-researched as a feedstock for bioenergy production because of their low lignin content (Chen et al., 2022). While it is possible to produce bioethanol, biohydrogen, and bio-oil from the plant, the most viable strategy for energy recovery from duckweed is the production of biogas via anaerobic digestion (Calicioglu and Brennan, 2018; Rana et al., 2021). This concept has been well-researched with many studies evaluating the plant as a co-substrate for mesophilic (35 ºC) anaerobic co-digestion combined with feedstocks such as animal manure, food waste, and wastewater (Gaur et al., 2017; Gaur and Suthar, 2017; Navarro et al., 2022; Panpong et al., 2014; Pena et al., 2017; Toyama et al., 2018). All of such studies came to the conclusion that the addition of duckweed to the anaerobic digestion of the primary substrate increased methane yeilds. Primary knowledge gaps in the field of duckweed-based biogas production remain in the anaerbic digestion of the plant under thermophilic (55 ºC) conditions.

Matošević et al. (2019) conducted thermopilic anaerbic digestion of duckweed biomass via batch tests to evaluate the methane yield from duckweeds grown in media containing varying amounts of digestate. While specific methane yields were not provided, it was concluded that duckweed grown in media containing 0.428% digestate produced a relatively higher volume of methane. Calicioglu et al. (2018) used batch tests to evaluate the effects temerature and pH have on the microbiome of duckweed-fed acidogenic anaerobic digesters. Methane production was not the goal of this system and it was found that methanogenic activity was inhibited within the thermophilic acidic system by lower solids solubilization. Total methane yields were not comparable to biomethane production rates of previous reports. Ramaraj and Unpaprom (2016) utilized batch tests to compare room temperature, mesophilic, and thermophilic anaerobic digestion of duckweed biomass and concluded that the mesophilic reactors outperformed the others. Specific methane production rates in terms of volume methane produced per unit mass COD or VS were not provided, alternatively this conclusion was founded only on biogas volume differences: 10,380 mL and 9,981 mL from the mesophilic and thermophilic digesters, respecively. This is not sufficient information for the comparison of this study to others. To the knowledge of this researcher, a biomethane potential assay (BMP) of duckweed as a single-substrate for thermophilic anaerobic digestion has not been conducted and the reaction kinetics of such a system have not been investigated. This study is necessary for a comprehensive evaluation of duckweed as a feedstock for thermophilic anaerobic digestion.

Batch anaerobic digestion tests are used for two main reasons: first to determine the ultimate methane yield from a substrate (or BMP) and second to evaluate the kinetic processes within the reaction and specify governing parameters of kinetic equations. Kinetic modeling is a critical research area regarding anaerobic digestion because it enables the understanding of key parameters necessary for optimization, simulation, and scaling up a system (Pererva et al., 2020; Pramanik et al., 2019). Guar et al. (2017) and Navarro et al. (2022) both conducted kinetic modeling of anaerobic co-digestion systems using the modified Gompertz model. Both studies found the model fit to be sufficient for parameter viability, and neither study reviewed alternative model choices (Gaur et al., 2017; Navarro et al., 2022). For a complete kinetic evaluation of duckweed-based anaerobic digestion, such a study is necessary. This study aims to investigate batch thermophilic anaerobic digestion of duckweed as a single substrate. The experiment will yield biomethane potential results for three duckweed types and the complete results of the experiment will be used to fit four kinetic models (First Order Model, Modified Gompertz

Model, Transference Model, and Logistic Function Model) to investigate the suitability of the substrates.

## 3.2. Materials and Methods

### 3.2.1. Inoculum Preparation

Inocula for the batch digestions were collected from steady-state thermophilic laboratory CSTR digesters processing duckweed and swine wastewater at an organic loading rate of 2,000 mg COD $L^{-1}$ digester $day^{-1}$ at a HRT of 10 days. Inocula were referred to by the associated duckweed feedstock: Culbreth, Raleigh, or Sicily. Inocula were taken directly from stable continuous thermophilic (50 ºC) anaerobic digesters and placed within the associated batch reactors.

### 3.2.2. Lemnaceae Preparation

Dried duckweed was prepared via the same method described in Chapter 2. Briefly, grown and harvested at the Lake Wheeler Road Field Laboratory of North Carolina State University (Raleigh, NC) adjacent to the swine wastewater lagoon. The duckweed was grown in a 300 sq ft hydroponic system utilizing swine wastewater as a fertilizer and harvested twice per week. The harvested biomass was put into mesh bags and allowed to air dry for several days, then it was spread out to dry in the sun before being stored at a moisture content of 10-15%. Two of the three varieties chosen for this study were selected based on findings from previous research studying the plant's ability to sequester nutrients from swine wastewater (Bergmann et al., 2000b, 2000a). Accession numbers for these two varieties from the Rutgers Collection (RDSC, http://www.ruduckweed.org/) are Lg8678 and Lg7741 for the *Lemna Gibba* (Raleigh) and *Lemna Gibba* (Sicily), respectively. The third and final duckweed variety was found growing in a pond approximately 50 miles from the NC State University campus and was collected by the project's research technician Michael Adcock. This variety has not yet been genotyped at this time but it is theorized that it is of the family *Spirodela* and has the shorthand name Culbreth. The Lemna varieties obtained for this study will be referenced to by their shorthand names: Raleigh, Sicily, and Culbreth.

Dried duckweed biomass was collected and brought back to Weaver Laboratories at North Carolina State University (Raleigh, NC) where all subsequent experiments took place. Previous CSTR trials using whole dried duckweed biomass proved that mixing would be difficult as the plant continued to float within the digesters. In order to maintain consistent mixing within the reactors, the biomass was mechanically pretreated. The biomass was milled using a biomass mill (IKA MF10) with a 0.25 sieve (0.25 mm hole size) and stored in plastic bags at room temperature. Biomass Characteristics are shown in Table 3.1.

**Table 3.1.** Lemnaceae biomass characteristics.

| Parameter | Sicily | Culbreth | Raleigh |
|---|---|---|---|
| Total Kjeldahl Nitrogen (mg/L) | 43200 | 47720 | 44240 |
| Chemical Oxygen Demand (mg/kg) | 898500 | 913700 | 916100 |
| Total Phosphorus (mg/L) | 26470 | 14740 | 23470 |
| Total Solids (%) | 88.52 | 96.79 | 89.53 |
| Volatile Solids (%) | 74.84 | 84.39 | 75.89 |

### 3.2.3. Batch Reactor Operation

Standard procedure for biomethane potential tests (BMP) outlined by Holliger et al. (2016) were used for this study. ANKOM bioreactors (RF Gas Production System, Macedon, NY), fitted with automatic pressure sensing modules were used for the BMP with 400 mL working volume and 622 mL total volumes. The three duckweed varieties, their associated inoculum controls, and pure corn starch (positive control) reactors were tested in duplicate. Total volatile solids (VS) content of 20 g VS/L with a substrate VS to inoculum VS ratio of 1:1 (4 g VS from both inoculum and substrate) was used, in accordance with the standard procedure.

After the reactor contents were prepared, the media and headspace were purged for one minute each using nitrogen gas (99.99%) to ensure an absence of oxygen. The reactors were incubated at 50 ºC in temperature-controlled water baths. Headspace pressure was set to 3.3 psi as recommended by Yan et al.. The length of the batch digestion was not predetermined. The BMP continued until three consecutive days produced less than 1% of the total biogas produced, which for this experiment was 28 days.

### 3.2.4. Analytical Methods

Total solids (TS) and volatile solids (VS) analyses of the three inocula, three duckweed biomass samples, and corn starch samples were conducted according to standard methods (APHA, 1998). Gas composition samples were taken twice during the first week of the BMP and once during the second, third, and fourth weeks. Samples were analyzed using gas chromatography (GC) (Shimadzu GC-2014, Kyoto, Japan) with a thermal conductivity detector and equipped with a 3 m by 3 mm 100/120 Carbosieve SII packed column (Supelco, Bellefonte, Pennsylvania) with helium as the carrier gas.

### 3.2.5. Data Analysis

#### 3.2.5.1. BMP Assay

Biomethane potential for the experimental and control assays were calculated under standard conditions and expressed as volume of methane per unit mass volatile solids from the substrate (N mL $CH_4$ $g^{-1}$ VS or COD). ANKOM gas production systems measure gas pressure accumulated within and released from fermentation reactors. To convert pressure (psi) to gas volume at standard conditions (N mL) the Ideal gas law (Eq. 1) is utilized:

$$n = p\left(\frac{V}{RT}\right) \tag{1}$$

where n = gas produced in moles; p = pressure in kilopascal; V = volume of reactor headspace in liters; T = temperature in Kelvin; R = gas constant (8.314472 L kPa $K^{-1}mol^{-1}$). Avogadro's law was then used to convert moles of biogas to mL.

To validate the inocula used for this experiment, starch was chosen as the positive control. The theoretical biomethane potential of corn starch was calculated using a procedure outlined by several studies (Eq. 2) (Achinas et al., 2016; Li et al., 2018; Rodriguez-Chiang and Dahl, 2015). Briefly, the theoretical biomethane potential (TBMP) of a compound or feedstock can be calculated using the elemental composition of the biomass and the Buswell equation:

$$TBMP\ (ml\ CH_4\ gVS^{-1}) = \frac{22.4 \times \left(\frac{a}{2}+\frac{b}{8}-\frac{c}{4}-\frac{3d}{8}-\frac{e}{4}\right)}{12.017a + 1.0079b + 15.999c + 14.0067d + 32.065\ e} \tag{2}$$

where *a*, *b*, *c*, *d*, and *e* are the molar ratios of carbon, hydrogen, oxygen, nitrogen, and sulfur within a compound. Starch is glucopyranose units linked via α-d-glycosidic bonds and has the chemical formula $(C_6H_{10}O_5)_n$ (Cheng, 2018). Using the molar ratios of carbon, hydrogen, and oxygen of starch, the TBMP can be calculated to be 414.4 mL $CH_4$ $gVS^{-1}$.

### 3.2.5.1. Kinetic Study

Kinetic modeling was conducted in this study in which the cumulative methane production experimental data was used to fit existing biogas production models. Four models were selected to estimate anaerobic digestion performance parameters from the data collected in the experiment: first order, modified Gompertz, transference, and logistic function models. The first order kinetic model (Eq. 3) is a very conventional method for kinetic modeling and has been used for many years to predict maximum methane potential (mL $CH_4$ $g^{-1}$ VS) and the first-order rate constant, k ($day^{-1}$) , also referred to as the hydrolysis constant (Wang et al., 2021).

The First Order Model:

$$M(t) = M_u \times (1 - \exp(-kt)) \tag{3}$$

where M(t) is the cumulative methane (mL g-VS$^{-1}$) (represented by net methane) at digestion time t (day), $M_u$ is the ultimate methane yield (mL g-VS$^{-1}$), and k is the hydrolysis constant or first order rate constant (day$^{-1}$). While the first order kinetic model has been validated and used for many years, many suggest the use of additional models that elucidate details regarding methane generation within an anaerobic digester (Pererva et al., 2020). One such model is the modified Gompertz model (Eq. 4), which allows the parameterization of methane potentials, methane production rates, and lag times (time until biogas generation).

The Modified Gompertz Model:

$$M(t) = M_u \times exp\left(-\exp\left[\frac{R_m \times e}{M_u} \times (\lambda - t) + 1\right]\right) \tag{4}$$

where M(t), $M_u$, and t have the same definition and unit as defined in the first order model, $R_m$ is the maximum methane production rate (mL g-VS$^{-1}$day$^{-1}$) and λ is the length of the lag phase (day). The Transference Model (Eq. 5) is used to determine the same parameters as the modified Gompertz equation with the assumption that methane generation is only dependent on bacterial growth rates (Alqaralleh et al., 2016; Fernández-Rodríguez et al., 2022; Ugwu and Enweremadu, 2019).

The Transference Model:

$$M(t) = M_u\left(1 - \exp\left(-\frac{R_m(t-\lambda)}{M_u}\right)\right) \tag{5}$$

where M(t), $M_u$, t, $R_m$, and λ have the same definition as previously defined for the modified Gompertz model. The logistic function model (Eq. 6) also assumes governing microbial growth kinetics and predicts the same parameters as the transference and modified Gompertz equations (Pererva et al., 2020; Ugwu and Enweremadu, 2019).

The Logistic Function Model:

$$M(t) = \frac{M_u}{1+\exp\left[\frac{4*R_m}{Mu}*(t-\lambda)+2\right]} \tag{6}$$

where M(t), $M_u$, t, $R_m$, and λ have the same meanings as previously defined. Model fitness can be quickly judged by $R^2$ and adjusted-$R^2$ values (a value closer to 1 being preferred), however, there are several other criteria that can determine goodness of fit for a kinetic model (Pererva et al., 2020). This study will look at residual sum of squares (RSS) and root mean square error (RMSE) which are quantities that can be generated via the modeling software. This study also calculated the corrected Akaike Information Criterion (AICc) and Bayesian Information Criterion (BIC) which are two criteria used to reduce overfitting in the model, where lower values indicate a better fitting model (Y. Li et al., 2018; Pererva et al., 2020). AICc and BIC formulas are detailed in Eq. 7 and Eq. 8:

$$AICc = N \times \ln\left(\frac{RSS}{N}\right) + 2K + \frac{2K(K+1)}{N-K-1} \tag{5}$$

$$BIC = \ln\left(\frac{RSS}{N}\right) + K \times \ln(N) \tag{5}$$

where N is the number of data points, RSS is the residual sum of squares, and K is the number of parameters the model is used to fit plus one (Y. Li et al., 2018). Origin software was used for the kinetic modeling of the batch digestion experiment (OriginLab Corporation, MA, USA) and Excel was used for calculations of criteria.

## 3.3. Results and Discussion

### 3.3.1. Biomethane Potential Test

Daily cumulative biogas and methane measurements for the 28-day batch digestion experiment are shown in Tables A.2.1 – A.2.3. Standard BMP procedure culminated in methane production results described as mL $CH_4$ $g^{-1}$ $VS_{fed}$, however, this study also reviewed methane production as mL $CH_4$ $g^{-1}$ $COD_{fed}$ because COD is a more representative measurement of available organics within the substrate (Meegoda et al., 2018). To conform to the BMP standard as well as previous studies in this area, results will be presented and discussed in terms of both $VS_{fed}$ and $COD_{fed}$. All results going forward will be discussed in terms of net biogas accumulated. Net biogas and net methane are used as the measurements for biomethane potential and represent the volume of biogas/methane produced by each inoculum-control subtracted from the volume produced by the corresponding experimental reactors. The positive control, corn starch, displayed BMPs of 371.4, 351.9, and 405.0 mL $CH_4$ $g^{-1}$ VS for the Culbreth, Raleigh, and Sicily inocula, respectively, which correspond to 90%, 85%, and 97% of the theoretical biomethane potential. These values are within the range of 85% - 100% specified as acceptable by the standard procedure and validate the activity of the three inocula in the BMP and batch digestion (Holliger et al., 2016).

Figure 3.1. displays the biogas and methane production curves (mL $g^{-1}$ $VS_{fed}$) as volume was accumulated throughout the 28-day batch test. The final methane production rates, or BMPs, for the three duckweed types were 205, 217, and 262 mL $CH_4$ $g^{-1}$ $VS_{fed}$ for the Culbreth, Raleigh, and Sicily duckweed types, respectively. ANOVA tests resulted in p-values listed in Table 3.2., and supported the conclusion that there was a significant ($p = 0.0084$, $p = 0169$) difference between the methane production of the Sicily duckweed compared to the other two types (which

did not show significant difference). The Sicily duckweed produced significantly more methane per unit mass VS fed. The BMP of 262 mL $CH_4$ $g^{-1}$ VS is comparable to the range of 190 – 340 mL $CH_4$ $g^{-1}$ VS obtained from the review of previous literature (Cu et al., 2015; Tonon et al., 2017). This result is also comparable to those produced by experiments conducting alkaline and fermentation pretreatments (190 and 230 mL $CH_4$ $g^{-1}$ VS, respectively) and co-digestion of duckweed with food waste (232 mL $CH_4$ $g^{-1}$ VS) (Navarro et al., 2022; Tonon et al., 2017). The BMP determined in this batch experiment was lower than those obtained during the co-digestion of duckweed with concentrated municipal and swine wastewaters (340 and 361 mL $CH_4$ $g^{-1}$ VS, respectively) and thermally pre-treated waste activated sludge (468 mL $CH_4$ $g^{-1}$ VS) (Gaur et al., 2017; Toyama et al., 2018). This supports the claims that duckweed often performs better as a co-substrate (depending on the composition of the secondary feedstock) for anaerobic digestion, and that there are available pretreatment strategies to enhance existing methane generation (Buragohain et al., 2021; Chen et al., 2022; Gaur et al., 2017; Navarro et al., 2022).

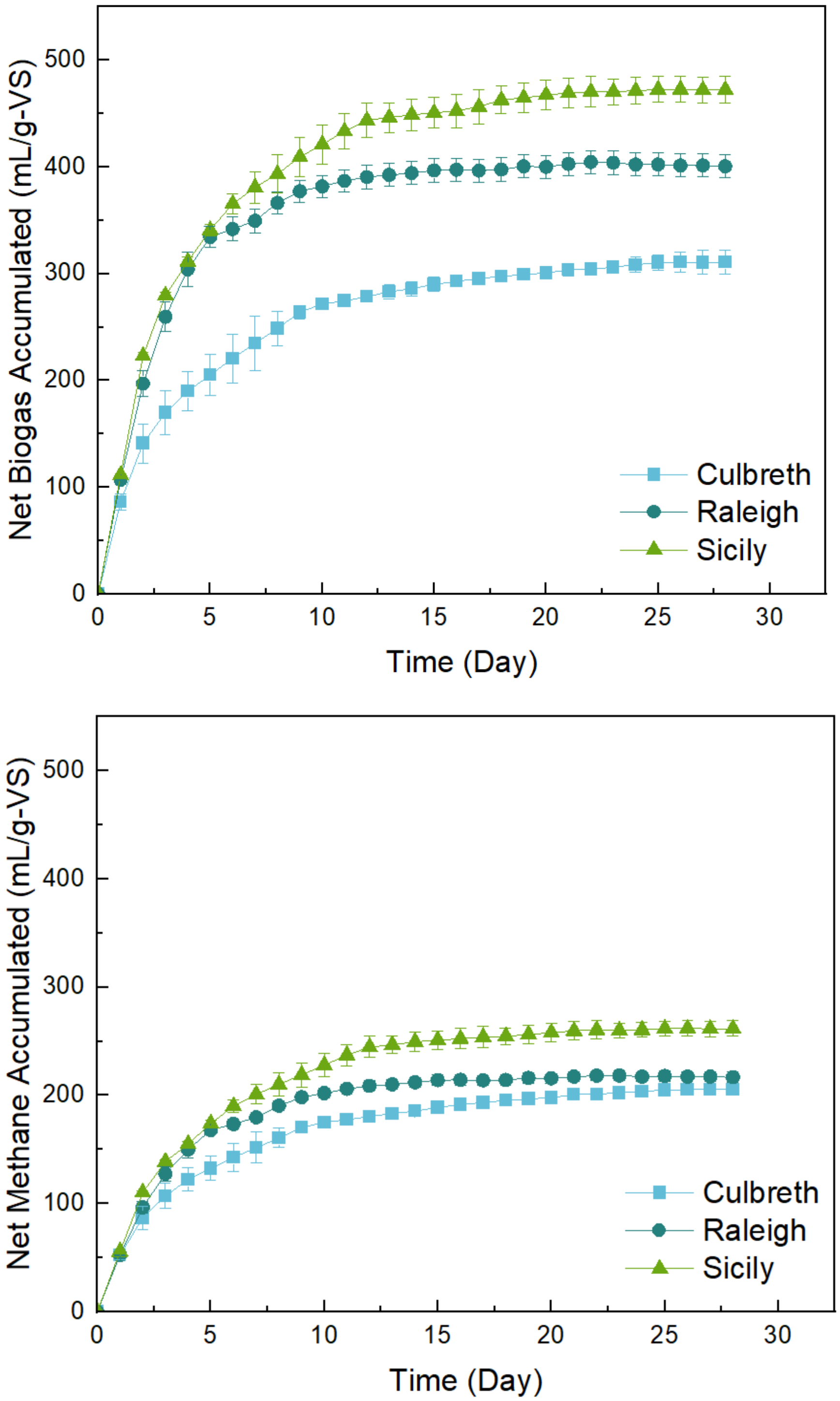

**Figure 3.1.** Biogas and methane production curves (VS). Net biogas (top) and methane (bottom) accumulated (mL $g^{-1}$ $VS_{fed}$) by each duckweed type during the 28-day batch test.

**Table 3.2.** One way ANOVA test p-values of net biogas/methane (mL $g^{-1}$ VS) produced during experiment.

| **Lemna Type** | **Culbreth** | **Raleigh** | **Sicily** |
|---|---|---|---|
| **Culbreth** | 1.0000/1.0000 | 0.0200/0.2100 | 0.0080/0.0084 |
| **Raleigh** | | 1.0000/1.0000 | 0.0112/0.0169 |
| **Sicily** | | | 1.0000/1.0000 |

Biogas production curves showing the daily net biogas and methane volumes (mL $g^{-1}$ $COD_{fed}$) for the three duckweed types evaluated in the batch digestion experiment are shown in Figure 3.2.. The final methane production rates from the BMP experiment were 188, 172, and 193 mL $g^{-1}$ $COD_{fed}$ for the Culbreth, Raleigh, and Sicily duckweed types, respectively. The p-value results from one-way ANOVA testing conducted on the biogas and methane final volumes in terms of $COD_{fed}$ are listed in Table 3.3.. These three BMP values should not be compared because the batch reactors had different loadings in terms of mg COD per L. The methane potentials determined by the BMP are in agreement with those calculated in Chapter 2, which were 0.17 – 0.19 mL $CH_4$ $g^{-1}$ $COD_{fed}$, 0.17 – 0.19 mL $CH_4$ $g^{-1}$ $COD_{fed}$, and 0.16 – 0.19 mL $CH_4$ $g^{-1}$ $COD_{fed}$ for the Lapp, Rapp, and BioFlo continuous reactors, respectively.

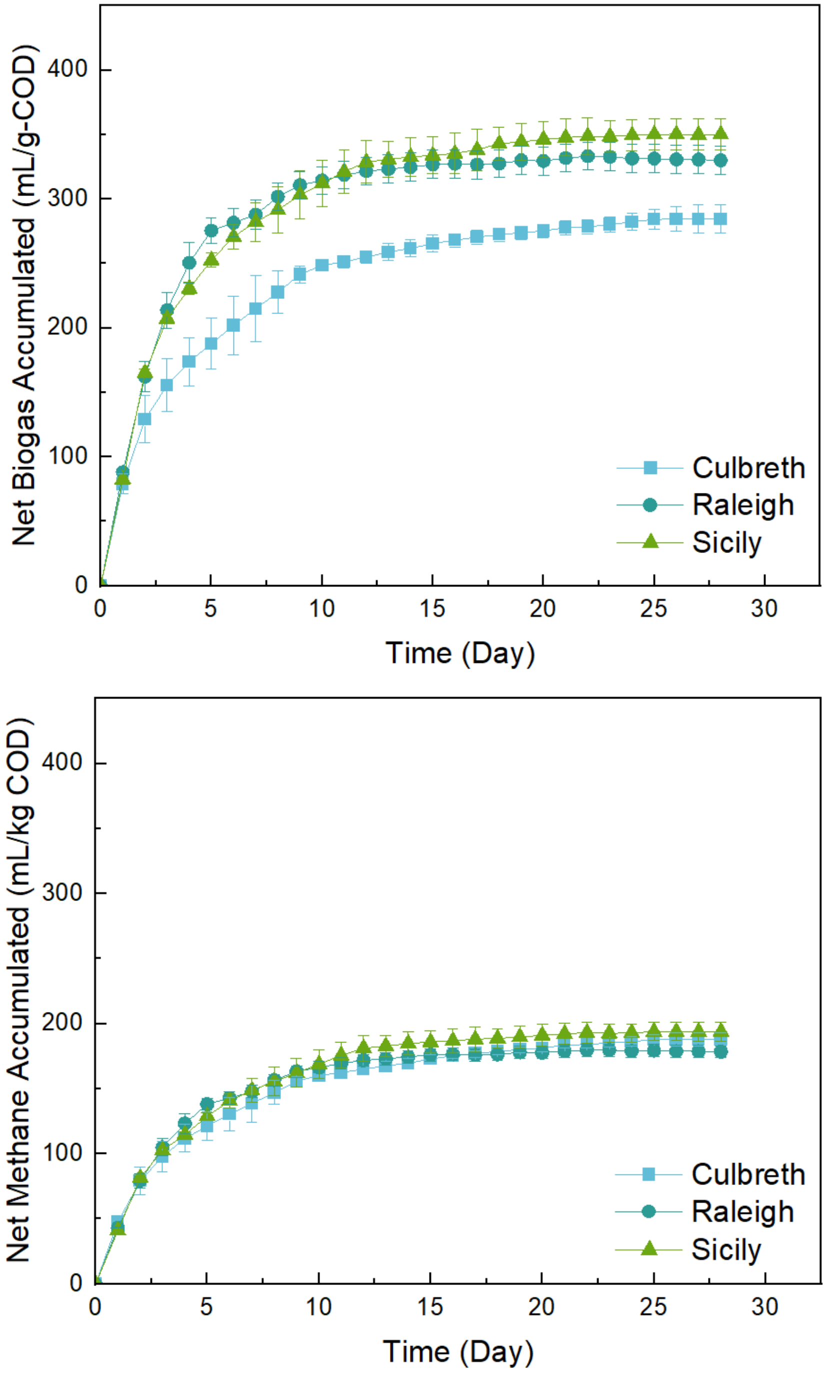

**Figure 3.2.** Biogas and methane production curves (COD). Net biogas (top) and methane (bottom) accumulated (mL $g^{-1}$ $COD_{fed}$) by each duckweed type during the 28-day batch test.

**Table 3.3.** One way ANOVA test p-values of net biogas/methane (mL $g^{-1}$ $COD_{fed}$) produced during experiment.

| **Lemna Type** | **Culbreth** | **Raleigh** | **Sicily** |
|---|---|---|---|
| **Culbreth** | 1.0000/1.0000 | 0.0114/0.2152 | 0.0045/0.1711 |
| **Raleigh** | | 1.0000/1.0000 | 0.1355/0.0643 |
| **Sicily** | | | 1.0000/1.0000 |

Table 3.4. displays a summary of all results from the BMP batch test from both VS and COD orientations. COD is a more comprehensive measurement of the organics within a feedstock thane VS, which represents the organic fraction of total solids (Meegoda et al., 2018). The biological oxygen demand (BOD) would be the most accurate parameter of digester effectiveness as it represents all biodegradable organics within the substrate. However, testing BOD is time consuming as it relies on bacteria, so it is not frequently practiced in lab scale anaerobic digestion experiments. COD is larger than BOD because it includes nonbiodegradable organics, but it is still a more accurate representation of a substrate than VS (Meegoda et al., 2018). Comparing the results from the batch digestion, it is more likely that the conclusion determined from the COD-based route is more accurate: that there was no statistical difference between the duckweed types in terms of methane production potential.

**Table 3.4.** Summary of BMP experiment.

| **Sample** | **Sample Loading (g VS/L)** | **Sample Loading (g COD/L)** | **Final Volume Biogas (mL)** | **Final Volume Methane (mL)** | **Biogas Yield (mL/g VS)** | **Methane Yield (mL/g VS)** | **Biogas Yield (mL/g COD)** | **Methane Yield (mL/g COD)** |
|---|---|---|---|---|---|---|---|---|
| Culbreth Biomass | 10.0 | 11.0 | 1243 | 821.3 | 310.8 | 205.3 | 284.6 | 188.0 |
| Raleigh Biomass | 10.0 | 12.6 | 1602 | 866.6 | 400.5 | 216.7 | 318.0 | 172.0 |
| Sicily Biomass | 10.0 | 13.5 | 1890 | 1047 | 472.4 | 261.6 | 354.3 | 192.8 |

### 3.3.2. Kinetic Study and Model Selection

This study reviewed the fit of the first order, modified Gompertz, transference and logistic function models to evaluate the performance of the batch test and model the anaerobic digestion of duckweed biomass. Figures 3.3. – 3.5. display the cumulative methane production rates (mL $CH_4$ $g^{-1}$ VS) for the three duckweed types with the fitted models.

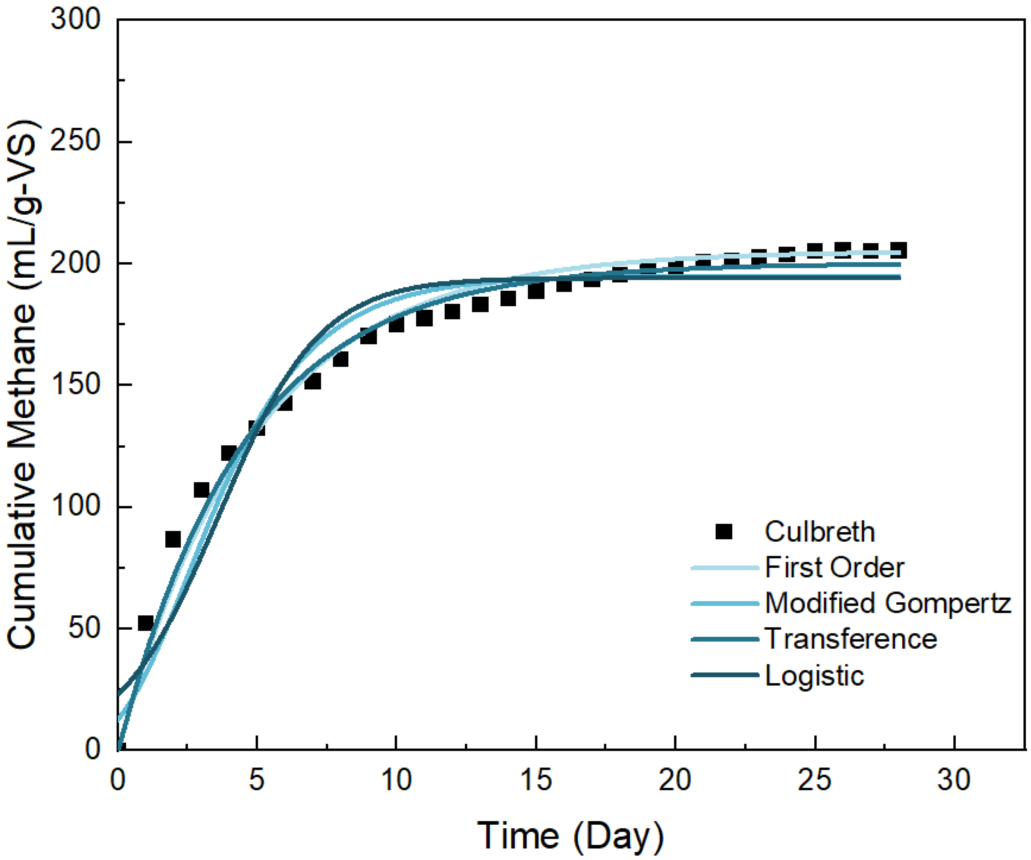

**Figure 3.3.** Kinetic modeling results for Culbreth methane production. First order, modified Gompertz, transference, and logistic models fitted to the experimental data associated with the Culbreth duckweed group.

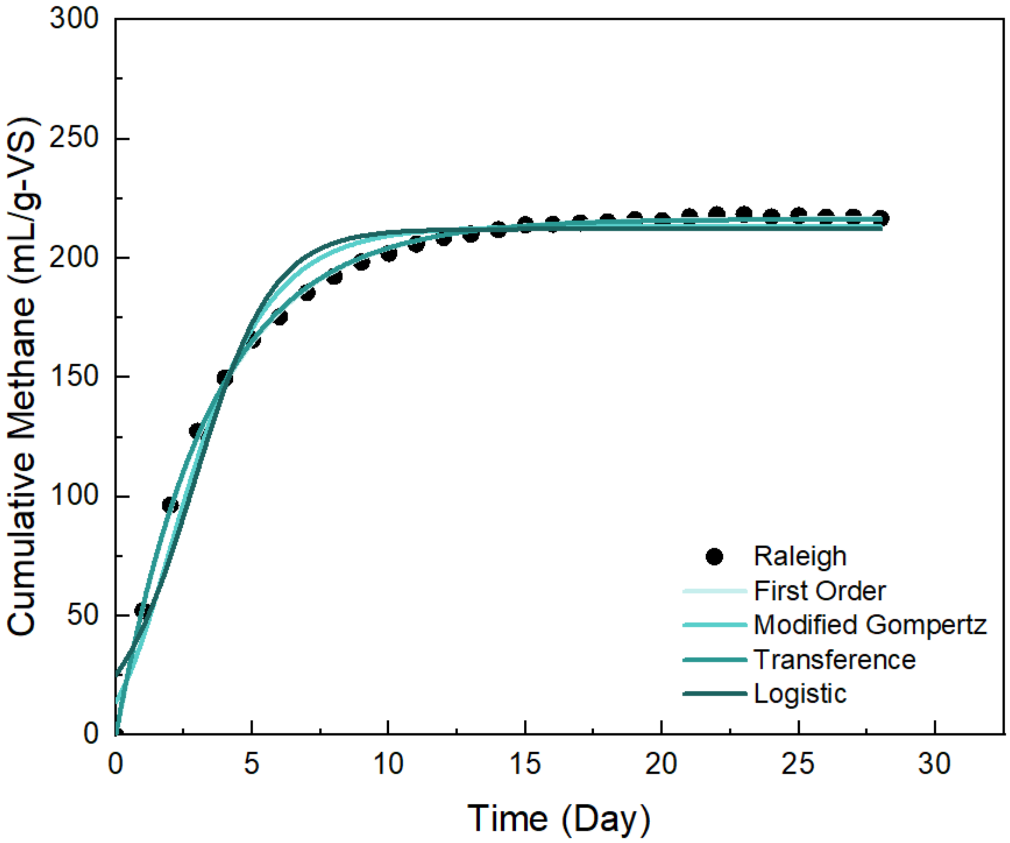

**Figure 3.4.** Kinetic modeling results for Raleigh methane production. First order, modified Gompertz, transference, and logistic models fitted to the experimental data associated with the Raleigh duckweed group.

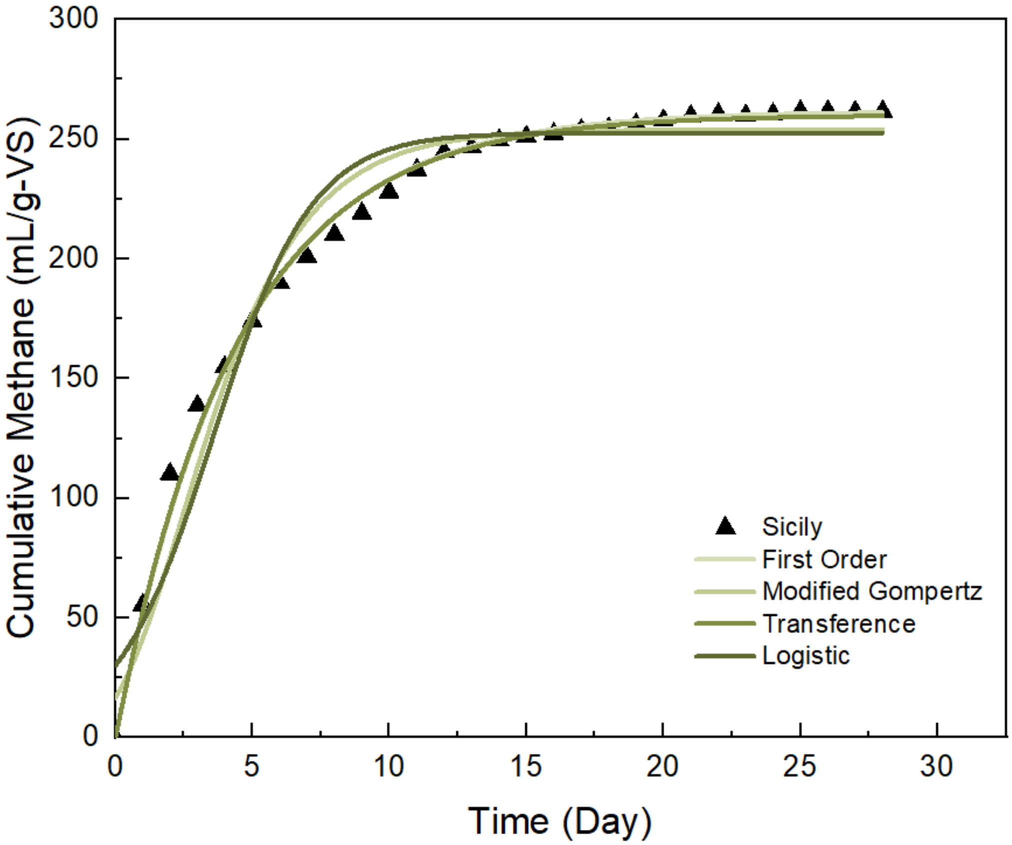

**Figure 3.5.** Kinetic modeling results for Sicily methane production. First order, modified Gompertz, transference, and logistic models fitted to the experimental data associated with the Sicily duckweed group.

Table 3.5. lists the parameters fitted from the four models including biogas production potential, first order rate constant, lag time, maximum biogas production rate, and the associated $R^2$ and adjusted-$R^2$. Lag phase in the modified Monod, transference, and logistic models was set to zero because there was no observed lag time. Lag time would be a necessary parameter in studies using an unacclimated inoculum. This experiment used inoculum directly from steady-state anaerobic digesters processing this feedstock at this temperature, so there was no lag phase. The differences between the predicted and observed methane productions for the three duckweed feedstocks were very small (0.03 – 5.43%) for all kinetic models. This indicates that all four models can be used to accurately estimate the methane produced from duckweed biomass

(Pramanik et al., 2019). All models displayed a reasonably good fit based on the adjusted-$R^2$ results. The first order kinetic model had the best estimation of the experimental data with an adjusted-$R^2$ value greater than 0.99 for all three duckweed types. This is the same conclusion determined by Kafle and Chen (2016) in their evaluation of five different manure streams for BMP. The transference model also displayed a suitable fit in this study for all three duckweed types with >0.98 adjusted-$R^2$ results. The first order and transference models also predicted the closest maximum methane production to the observed value. Ugwu and Enweremadu (2019) came to a similar conclusion that the transference and first order kinetic models supported better fits than the modified Gompertz in their investigation of okra waste anaerobic digestion. The hydrolysis rate constants determined by the first order kinetic model were 0.205, 0.285, 0.222 $day^{-1}$ for the Culbreth, Raleigh, and Sicily duckweed types, respectively. These results fit within the range observed in the literature of 0.1196 – 0.43 for manure-based anaerobic digestion systems (Kafle and Chen, 2016; Ma et al., 2013; Mao et al., 2017). The average hydrolysis constant determined in this study (0.237) was similar to that determined by Ugwu and Enweremadu (2019) who processed okra with a first order constant of 0.29. This result reflects high degradation of the duckweed biomass (Ugwu and Enweremadu, 2019). Further evaluation of the model accuracy can confirm this conclusion.

**Table 3.5.** Summary of kinetic modeling parameters.

| Model | Parameter | | Unit | Duckweed Type | | |
|---|---|---|---|---|---|---|
| | | | | **Culbreth** | **Raleigh** | **Sicily** |
| First Order | Methane Yield ($M_u$) | Measured | mL g$^{-1}$ VS$_{fed}$ | 205.31 | 216.66 | 261.62 |
| | | Predicted | mL g$^{-1}$ VS$_{fed}$ | 204.65 | 216.59 | 261.10 |
| | | Δ | % | 0.32 | 0.03 | 0.20 |
| | Rate Constant (k) | | Day$^{-1}$ | 0.205 | 0.285 | 0.222 |
| | $R^2$ | | | 0.993 | 0.999 | 0.995 |
| | Adjusted $R^2$ | | | 0.993 | 0.999 | 0.995 |
| Modified Gompertz | Ultimate Methane ($M_u$) | Measured | mL g$^{-1}$ VS$_{fed}$ | 205.31 | 216.66 | 261.62 |
| | | Predicted | mL g$^{-1}$ VS$_{fed}$ | 194.94 | 213.22 | 254.06 |
| | | Δ | % | 5.05 | 1.59 | 2.89 |
| | Ultimate Methane Production Rate ($R_m$) | | mL $CH_4$ g$^{-1}$ VS$_{fed}$ Day$^{-1}$ | 28.797 | 39.272 | 37.759 |
| | Lag Time (λ) | | Day | 0 | 0 | 0 |
| | $R^2$ | | | 0.948 | 0.977 | 0.967 |
| | Adjusted $R^2$ | | | 0.946 | 0.977 | 0.966 |
| Transference | Methane Yield ($M_u$) | Measured | mL g$^{-1}$ VS$_{fed}$ | 205.31 | 216.66 | 261.62 |
| | | Predicted | mL g$^{-1}$ VS$_{fed}$ | 200.14 | 216.40 | 260.13 |
| | | Δ | % | 2.52 | 0.12 | 0.57 |
| | Ultimate Methane Production Rate ($R_m$) | | mL $CH_4$ g$^{-1}$ VS$_{fed}$ Day$^{-1}$ | 44.330 | 62.585 | 58.790 |
| | Lag Time (λ) | | Day | 0 | 0 | 0 |
| | $R^2$ | | | 0.987 | 0.986 | 0.995 |
| | Adjusted $R^2$ | | | 0.986 | 0.986 | 0.995 |
| Logistic | Methane Yield ($M_u$) | Measured | mL g$^{-1}$ VS$_{fed}$ | 205.31 | 216.66 | 261.62 |
| | | Predicted | mL g$^{-1}$ VS$_{fed}$ | 194.17 | 212.21 | 252.62 |
| | | Δ | % | 5.43 | 2.05 | 3.44 |
| | Ultimate Methane Production Rate ($R_m$) | | mL $CH_4$ g$^{-1}$ VS$_{fed}$ Day$^{-1}$ | 26.744 | 36.945 | 35.205 |
| | Lag Time (λ) | | Day | 0 | 0 | 0 |
| | $R^2$ | | | 0.965 | 0.997 | 0.951 |
| | Adjusted $R^2$ | | | 0.965 | 0.997 | 0.949 |

The estimated methane yield and $R^2$ values are not the only parameters necessary to judge the goodness of fit for each kinetic model. To conduct a deeper investigation of the kinetic models, this study utilized the procedure describes in previous literature and also reviewed statistical indicators including root mean square error (RMSE), residual sum of squares (RSS) and the Akaike Information Criterion (AICc) and Bayesian Information Criterion (BIC) (see Table 3.6.) (Y. Li et al., 2018; Pramanik et al., 2019). For all of the evaluated criteria, a lower value is preferred. The first order, transference, and logistic models had RMSE < 10 for all three duckweed types, which supports a conclusion that they are close predictors for model parameters (Yono et al., 2014). The RMSE, RSS, AICc, and BIC values were the lowest for the first order kinetic model for the Raleigh and Culbreth duckweed groups. This was followed by, in ascending order, the transference model, the logistic model, and the modified Gompertz model. Note that the RMSE for the logistic model produced from the Raleigh data was lower than that of the transference model, but the RSS AICc and BIC values were lower for the latter. The Sicily duckweed data was best predicted by the transference model, followed by the first order kinetic model, the modified Gompertz, and lastly the logistic model. The results, broadly, concluded that the transference and first order models supported the closest fit to the experimental data. This result strengthens the previous claim that these two models had the closest fits to the observed data, and supports the previous conclusions made regarding the degradability of the duckweed biomass.

**Table 3.6.** Analysis of fit criteria for kinetic models.

| Model | Statistical Indicator | Duckweed Type | | |
|---|---|---|---|---|
| | | **Culbreth** | **Raleigh** | **Sicily** |
| First Order | RMSE | 3.270 | 1.455 | 4.654 |
| | RSS | 682.7 | 59.30 | 606.5 |
| | $AIC_c$ | 93.75 | 25.21 | 92.63 |
| | BIC | 94.971 | 27.480 | 94.904 |
| Modified Gompertz | RMSE | 11.730 | 5.817 | 12.292 |
| | RSS | 3715.07 | 3771.07 | 4079.76 |
| | $AIC_c$ | 142.88 | 145.63 | 147.91 |
| | BIC | 144.10 | 147.90 | 150.18 |
| Transference | RMSE | 5.878 | 5.573 | 4.636 |
| | RSS | 932.97 | 995.16 | 580.22 |
| | $AIC_c$ | 102.81 | 104.68 | 89.04 |
| | BIC | 104.03 | 109.27 | 93.62 |
| Logistic | RMSE | 7.51 | 2.54 | 15.07 |
| | RSS | 5263.21 | 6438.80 | 6130.29 |
| | $AIC_c$ | 152.98 | 158.83 | 157.41 |
| | BIC | 154.20 | 163.42 | 161.99 |

Previous studies that conducted the kinetic modeling of duckweed-based anaerobic digestion used secondary feedstocks (waste activated sludge and food waste materials) and only reviewed the modified Gompertz model. Navarro et al. (2022) found that the modified Gompertz model had a very close fit ($R^2 \geq 0.99$) to their experimental data from duckweed and food waste co-digestion, which reported a much larger methane production than this study (approximately 360 mL $CH_4$ $g^{-1}$ VS). However, Navarro et al. reported a much lower maximum methane production rate of approximately 15 mL $CH_4$ $g^{-1}$ VS $day^{-1}$ compared to 55 mL $CH_4$ $g^{-1}$ VS $day^{-1}$ observed in this study. This difference could possibly be attributed to the much longer duration of the Navarro et al. BMP (55 days) which was likely necessary for the completion of a mesophilic BMP. Whereas, the BMP batch test conducted in this study was performed at thermophilic temperature which is conducive to faster degradation and biological reactions, and enabled the BMP to be completed in 28 days. Gaur and Suthar (2017) reported lower $R^2$ results from their use of the modified Gompertz kinetic model (0.90 – 0.94) but still concluded that the fit was adequate enough to validate experimental data and choose an experimental group with the highest methane production. The methane yields reported by Gaur and Suthar from the modified Gompertz equation were 228, 237, 253, and 311 mL $CH_4$ $g^{-1}$ $VS_{removed}$, which are similar to the production rates observed in this batch test and those estimated from the transference model used in this study. Seeing that neither of the previous studies evaluating the kinetics of duckweed-based anaerobic digestion attempted to use/compare other models, it is impossible to know if the modified Gompertz was in fact the closest predictor to experimental data. To the knowledge of this researcher, there have not been comprehensive kinetic modeling studies evaluating the anaerobic digestion of duckweed biomass.

### 3.4. Conclusion

A batch test was conducted using duckweed biomass and acclimated thermophilic anaerobic digestate (inoculum) for 28 days in order to measure the biomethane potential and estimate the kinetic modeling parameters for three duckweed types: *Lemna gibba* (Raleigh), *Lemna gibba* (Sicily), and *Spirodela* (Culbreth). The BMPs were found to be 205, 217, and 262 mL $CH_4$ $g^{-1}$ $VS_{fed}$, or 188, 172, and 192 mL $CH_4$ $g^{-1}$ $COD_{fed}$ for the Culbreth, Raleigh and Sicily duckweed types, respectively. It was concluded, from the COD-based results, that the duckweed groups did not have significantly different methane potentials in the batch test. The methane production rates determined by the batch digestion agreed with those that resulted from the continuous reactor. This conclusion reiterates the value of running continuous anaerobic digesters as well as batch test experiments to determine conversion efficiencies of anaerobic systems.

Kinetic modeling using the first order, modified Gompertz, transference, and logistic function kinetic models was conducted using modeling software to determine which models suitably fit the experimental data. For the three duckweed groups, the first order and transference models had the best fits to the experimental data (based on $R^2$, RMSE, RSS, AICc, and BIC criteria). The first order model predicted the hydrolysis constant (k) to be 0.205 – 0.285 $day^{-1}$. This result is similar to the hydrolysis constants reviewed in the literature regarding effective anaerobic digestion systems, indicating that the duckweed biomass has viable degradation rates (Ugwu and Enweremadu, 2019).

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# CHAPTER 4: CONCLUSIONS AND FUTURE RESEARCH

## 4.1. Conclusions

The objective of this research project was to conduct and understand thermophilic anaerobic digestion of Lemnaceae, or duckweed, biomass for biogas production. To do so, batch and continuous scale anaerobic digestion experiments were performed and provided a comprehensive evaluation of the viability of the proposed system from the small (reaction kinetics) to large scales (continuous digestion). This study did not agree with previous literature in this area that concluded that thermophilic anaerobic digestion of duckweed was less productive than mesophilic (Ramaraj and Unpaprom, 2016; Tonon et al., 2017).

In this study, continuous thermophilic digestion of duckweed biomass combined with swine wastewater at a 10 day HRT resulted in a maximum methane yield of 0.362 $m^3$ $CH_4$ $kg^{-1}$ $COD_{consumed}$ from the *Spirodela* (Culbreth) duckweed type at the maximum organic loading rate evaluated, which was 4,500 mg COD $L^{-1}$ digester $day^{-1}$. This result was much higher than a similar study using the same substrates at lower organic loading rates with a mesophilic operating condition (Pena et al., 2017). This difference is likely due to the lower organic loading rate evaluated by Pena et al.. The results of this study were similar to those determined by Panpong et al. (2014) from a combined canned seafood wastewater and duckweed mesophilic digestion system, which was 0.352 $m^3$ $CH_4$ $kg^{-1}$ $COD_{removed}$. Therefore, thermophilic co-digestion of duckweed resulted in similar yields to its mesophilic counterpart, and is a viable pathway for biogas production from the biomass. Thermophilic can also be conducted at a shorter HRT, and therefore has the potential to process more biomass (Labatut et al., 2014).

Batch testing of duckweed biomass with acclimated thermophilic inocula resulted in BMP values validated by popular kinetic models. BMPs of 205, 217, and 262 mL $CH_4$ $g^{-1}$ $VS_{fed}$, or 188, 172, and 192 mL $CH_4$ $g^{-1}$ $COD_{fed}$ for the Culbreth, Raleigh and Sicily duckweed types, respectively, were determined, and these results were in agreement with the methane production rate of the continuous co-digesters. The first order and transference kinetic models fit the best to the experimental data and provided estimates for the hydrolysis constant (k), methane production ($M_u$), and maximum methane production rate ($R_m$). These values were 0.205, 0.222, and 0.285 $day^{-1}$, 205, 217, and 260 mL $CH_4$ $g^{-1}$ $VS_{fed}$, and 29, 39, and 38 mL $CH_4$ $g^{-1}$ $VS_{fed}$ $day^{-1}$ for the Culbreth, Raleigh, and Sicily duckweed types, respectively. The hydrolysis rate constant and methane production parameter estimations were similar to those produced from studies evaluating duckweed and swine manure and indicated successful degradation of the duckweed biomass (Gaur and Suthar, 2017; Mao et al., 2017). The methane production rate estimations (mL $CH_4$ $g^{-1}$ $VS_{fed}$ $day^{-1}$), however, were higher than studies evaluating similar substrates. This can be attributed to the faster rate of biogas production at the higher temperature. The batch tests performed in this study further proved the viability of duckweed biomass for thermophilic anaerobic digestion.

### 4.2 Future Research Recommendations

Recommendations for future research in this area are focused on continuous thermophilic anaerobic digestion and are both applied and fundamental. Firstly, it was observed that the methane yields produced in this study continued to increase with increasing OLR, so it is rational for the operational next step to be to continue increasing the OLR until a maximum methane yield is produced (which is theoretically inevitable) (Rincón et al., 2006). Secondly, the viability of the system designed, operated, and evaluated in this project proves the necessity of a deeper investigation into duckweed-based anaerobic digestion. This investigation should be conducted at the genomic level through a metagenomic and transcriptomic analysis of the microbial community within the microbiome of a duckweed-based thermophilic anaerobic digester. By characterizing the microbiome of anaerobic digesters with duckweed as the feedstock, one would understand what the microbial population is and how it compares to previously-sequenced microbiomes. This will highlight unique mechanisms within the anaerobic digesters. It has been shown through the evaluation of the microbiomes of digesters with conventional feedstocks that identification of functional groups, dynamics, and stability mechanisms creates a foundation for better-engineered systems (Werner et al., 2011). The identification of key microbes and bacterial interactions to digestion of duckweed will provide necessary support to further enhance or supplement the microbiome to promote stable renewable energy production.

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# APPENDICES

### A.1. Continuous Digestion Data, Daily biogas production (mL) and pH measurements.

| Date | Loading Rate | Daily Biogas Produced (mL) | | | pH Measurement | | |
|---|---|---|---|---|---|---|---|
| | | Rapp | Lapp | BioFlo | Rapp | Lapp | BioFlo |
| 8/27/2022 | Control | 3780 | 3525 | 0 | 7.3 | 7.3 | 7.3 |
| 8/28/2022 | Control | 2800 | 2350 | 1061 | 7.3 | 7.3 | 7.3 |
| 8/29/2022 | Control | 2520 | 2115 | 943 | 7.3 | 7.3 | 7.3 |
| 8/30/2022 | Control | 2520 | 2115 | 943 | 7.3 | 7.3 | 7.3 |
| 8/31/2022 | Control | 2240 | 2115 | 1061 | 7.3 | 7.3 | 7.3 |
| 9/1/2022 | Control | 1960 | 1645 | 943 | 7.3 | 7.3 | 7.3 |
| 9/2/2022 | Control | 2940 | 2115 | 118 | 7.3 | 7.3 | 7.3 |
| 9/3/2022 | Control | 840 | 470 | 118 | 7.3 | 7.3 | 7.3 |
| 9/4/2022 | Control | 560 | 470 | 118 | 7.4 | 7.3 | 7.3 |
| 9/5/2022 | Control | 280 | 470 | 118 | 7.4 | 7.3 | 7.2 |
| 9/6/2022 | Control | 280 | 470 | 118 | 7.3 | 7.3 | 7.2 |
| 9/7/2022 | Control | 420 | 705 | 118 | 7.4 | 7.4 | 7.2 |
| 9/8/2022 | Control | 840 | 940 | 354 | 7.4 | 7.3 | 7.3 |
| 9/9/2022 | Control | 700 | 1175 | 1414 | 7.3 | 7.5 | 7.1 |
| 9/10/2022 | Control | 420 | 1410 | 2121 | 7.1 | 7.4 | 7.3 |
| 9/11/2022 | Control | 560 | 2350 | 1061 | 7.2 | 7.5 | 7.5 |
| 9/12/2022 | Control | 1120 | 1410 | 1061 | 7.1 | 7.6 | 7.4 |
| 9/13/2022 | Control | 2380 | 1175 | 825 | 7.5 | 7.5 | 7.3 |
| 9/14/2022 | Control | 2800 | 1410 | 707 | 7.5 | 7.5 | 7.4 |
| 9/15/2022 | Control | 3220 | 1410 | 707 | 7.5 | 7.6 | 7.4 |
| 9/16/2022 | Control | 980 | 705 | 707 | 7.6 | 7.4 | 7.3 |
| 9/17/2022 | Control | 980 | 1175 | 707 | 7.3 | 7 | 7.2 |
| 9/18/2022 | Control | 1400 | 1645 | 1061 | 7.4 | 7.5 | 7.3 |
| 9/19/2022 | Control | 1260 | 1175 | 825 | 7.3 | 7.1 | 7.3 |
| 9/20/2022 | Control | 1260 | 1175 | 943 | 7.3 | 7.2 | 7.3 |
| 9/21/2022 | Control | 1400 | 1175 | 1061 | 7.3 | 7.4 | 7.3 |
| 9/22/2022 | Control | 1400 | 940 | 943 | 7.3 | 7.3 | 7.3 |
| 9/23/2022 | Control | 1680 | 1175 | 825 | 7.5 | 7.3 | 7.3 |
| 9/24/2022 | Control | 1120 | 940 | 825 | 7.5 | 7.3 | 7.2 |
| 9/25/2022 | Control | 1120 | 1410 | 1061 | 7.1 | 7.3 | 7.3 |
| 9/26/2022 | Control | 1400 | 940 | 707 | 7.2 | 7.4 | 7.3 |
| 9/27/2022 | Control | 1260 | 940 | 825 | 7.2 | 7.4 | 7.3 |
| 9/28/2022 | Control | 840 | 470 | 471 | 7.2 | 7.3 | 7.2 |
| 9/29/2022 | Control | 1400 | 1175 | 589 | 7.3 | 7.4 | 7.2 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 9/30/2022 | Control | 1260 | 940 | 707 | 7.4 | 7.5 | 7.4 |
| 10/1/2022 | Control | 560 | 705 | 354 | 7.4 | 7.3 | 7.2 |
| 10/2/2022 | Control | 980 | 940 | 825 | 7.4 | 7.5 | 7.3 |
| 10/3/2022 | Control | 1120 | 940 | 589 | 7.4 | 7.3 | 7.2 |
| 10/4/2022 | Control | 840 | 940 | 589 | 7.3 | 7.4 | 7.2 |
| 10/5/2022 | Control | 1540 | 1175 | 943 | 7.5 | 7.4 | 7.3 |
| 10/6/2022 | Control | 1680 | 1410 | 1179 | 7.4 | 7.5 | 7.2 |
| 10/7/2022 | Control | 1400 | 1645 | 1061 | 7.3 | 7.5 | 7.2 |
| 10/8/2022 | Control | 2240 | 1880 | 2004 | 7.4 | 7.5 | 7.5 |
| 10/9/2022 | Control | 1680 | 1175 | 1179 | 7.5 | 7.4 | 7.5 |
| 10/10/2022 | OLR-1 | 3220 | 2820 | 2121 | 7.4 | 7.4 | 7.5 |
| 10/11/2022 | OLR-1 | 3080 | 2820 | 2004 | 7.4 | 7.4 | 7.3 |
| 10/12/2022 | OLR-1 | 3640 | 2820 | 1650 | 7.4 | 7.3 | 7.4 |
| 10/13/2022 | OLR-1 | 3780 | 3055 | 1768 | 7.5 | 7.4 | 7.4 |
| 10/14/2022 | OLR-1 | 3500 | 2820 | 1886 | 7.3 | 7.4 | 7.5 |
| 10/15/2022 | OLR-1 | 3500 | 2820 | 2121 | 7.5 | 7.4 | 7.4 |
| 10/16/2022 | OLR-1 | 3220 | 3055 | 2121 | 7.5 | 7.4 | 7.5 |
| 10/17/2022 | OLR-1 | 3360 | 2820 | 2004 | 7.5 | 7.5 | 7.6 |
| 10/18/2022 | OLR-1 | 3080 | 2820 | 2004 | 7.5 | 7.5 | 7.5 |
| 10/19/2022 | OLR-1 | 3220 | 3055 | 2004 | 7.5 | 7.5 | 7.5 |
| 10/20/2022 | OLR-1 | 2940 | 2585 | 2357 | 7.5 | 7.5 | 7.5 |
| 10/21/2022 | OLR-1 | 3080 | 2820 | 2239 | 7.5 | 7.4 | 7.5 |
| 10/22/2022 | OLR-1 | 3360 | 2820 | 2357 | 7.5 | 7.5 | 7.6 |
| 10/23/2022 | OLR-1 | 3080 | 2820 | 2475 | 7.6 | 7.6 | 7.5 |
| 10/24/2022 | OLR-1 | 3220 | 2585 | 2475 | 7.5 | 7.5 | 7.6 |
| 10/25/2022 | OLR-1 | 2940 | 2820 | 2593 | 7.6 | 7.6 | 7.6 |
| 10/26/2022 | OLR-1 | 3080 | 2820 | 2593 | 7.6 | 7.5 | 7.6 |
| 10/27/2022 | OLR-1 | 3220 | 2585 | 2357 | 7.6 | 7.6 | 7.6 |
| 10/28/2022 | OLR-1 | 3080 | 2585 | 2239 | 7.5 | 7.5 | 7.7 |
| 10/29/2022 | OLR-1 | 2940 | 2820 | 2239 | 7.5 | 7.6 | 7.6 |
| 10/30/2022 | OLR-1 | 3080 | 2585 | 2239 | 7.6 | 7.6 | 7.6 |
| 10/31/2022 | OLR-1 | 3080 | 2820 | 2121 | 7.6 | 7.5 | 7.7 |
| 11/1/2022 | OLR-1 | 2240 | 1645 | 1179 | 7.7 | 7.5 | 7.7 |
| 11/2/2022 | OLR-2 | 2380 | 3995 | 4243 | 7.7 | 7.5 | 7.3 |
| 11/3/2022 | OLR-2 | 3080 | 3055 | 6129 | 7.6 | 7.4 | 7.6 |
| 11/4/2022 | OLR-2 | 3080 | 3525 | 6482 | 7.7 | 7.5 | 7.6 |
| 11/5/2022 | OLR-2 | 3080 | 2820 | 5539 | 7.7 | 7.6 | 7.6 |
| 11/6/2022 | OLR-2 | 3220 | 2820 | 3064 | 7.6 | 7.6 | 7.6 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 11/7/2022 | OLR-2 | 3640 | 3055 | 4243 | 7.6 | 7.6 | 7.6 |
| 11/8/2022 | OLR-2 | 2800 | 2585 | 4361 | 7.5 | 7.6 | 7.6 |
| 11/9/2022 | OLR-2 | 5460 | 4230 | 5186 | 7.5 | 7.4 | 7.5 |
| 11/10/2022 | OLR-2 | 5460 | 4465 | 5893 | 7.6 | 7.5 | 7.6 |
| 11/11/2022 | OLR-2 | 5180 | 3995 | 6129 | 7.6 | 7.7 | 7.6 |
| 11/12/2022 | OLR-2 | 5040 | 4230 | 6364 | 7.6 | 7.5 | 7.6 |
| 11/13/2022 | OLR-2 | 4340 | 3995 | 5539 | 7.6 | 7.6 | 7.7 |
| 11/14/2022 | OLR-2 | 4480 | 3760 | 5893 | 7.7 | 7.6 | 7.6 |
| 11/15/2022 | OLR-2 | 4620 | 3995 | 6482 | 7.7 | 7.5 | 7.6 |
| 11/16/2022 | OLR-2 | 4480 | 3995 | 6246 | 7.7 | 7.6 | 7.7 |
| 11/17/2022 | OLR-2 | 4480 | 4230 | 5775 | 7.6 | 7.5 | 7.6 |
| 11/18/2022 | OLR-2 | 4480 | 4465 | 5893 | 7.7 | 7.5 | 7.6 |
| 11/19/2022 | OLR-2 | 4900 | 4230 | 6246 | 7.7 | 7.6 | 7.5 |
| 11/20/2022 | OLR-2 | 4760 | 4230 | 6129 | 7.7 | 7.6 | 7.6 |
| 11/21/2022 | OLR-2 | 4760 | 3995 | 6364 | 7.7 | 7.5 | 7.6 |
| 11/22/2022 | OLR-2 | 4760 | 3995 | 6482 | 7.8 | 7.6 | 7.6 |
| 11/23/2022 | OLR-2 | 4620 | 3760 | 7543 | 7.7 | 7.6 | 7.5 |
| 11/24/2022 | OLR-2 | 4480 | 3760 | 7425 | 7.6 | 7.6 | 7.5 |
| 11/25/2022 | OLR-2 | 5460 | 4700 | 7779 | 7.7 | 7.7 | 7.6 |
| 11/26/2022 | OLR-2 | 5040 | 4230 | 7307 | 7.7 | 7.6 | 7.7 |
| 11/27/2022 | OLR-2 | 4900 | 4465 | 7189 | 7.8 | 7.6 | 7.6 |
| 11/28/2022 | OLR-2 | 5320 | 4465 | 7661 | 7.8 | 7.6 | 7.6 |
| 11/29/2022 | OLR-2 | 5180 | 4465 | 7425 | 7.7 | 7.7 | 7.6 |
| 11/30/2022 | OLR-3 | 4620 | 4230 | 7189 | 7.8 | 7.7 | 7.6 |
| 12/1/2022 | OLR-3 | 4900 | 4230 | 6954 | 7.7 | 7.7 | 7.6 |
| 12/2/2022 | OLR-3 | 4620 | 3995 | 7189 | 7.7 | 7.5 | 7.7 |
| 12/3/2022 | OLR-3 | 5740 | 4935 | 9900 | 7.6 | 7.5 | 7.5 |
| 12/4/2022 | OLR-3 | 6300 | 5640 | 11079 | 7.6 | 7.6 | 7.6 |
| 12/5/2022 | OLR-3 | 6160 | 5875 | 11196 | 7.6 | 7.6 | 7.5 |
| 12/6/2022 | OLR-3 | 9240 | 7285 | 12729 | 7.7 | 7.7 | 7.6 |
| 12/7/2022 | OLR-3 | 7140 | 5875 | 11904 | 7.7 | 7.7 | 7.6 |
| 12/8/2022 | OLR-3 | 6860 | 6345 | 11668 | 7.8 | 7.7 | 7.6 |
| 12/9/2022 | OLR-3 | 7700 | 7285 | 12846 | 7.7 | 7.7 | 7.6 |
| 12/10/2022 | OLR-3 | 7420 | 7050 | 13082 | 7.7 | 7.7 | 7.6 |
| 12/11/2022 | OLR-3 | 7980 | 7520 | 13436 | 7.7 | 7.8 | 7.6 |
| 12/12/2022 | OLR-3 | 8400 | 8225 | 13789 | 7.8 | 7.8 | 7.6 |
| 12/13/2022 | OLR-3 | 7700 | 7050 | 12964 | 7.8 | 7.8 | 7.6 |
| 12/14/2022 | OLR-3 | 8680 | 7285 | 13671 | 7.9 | 7.8 | 7.6 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 12/15/2022 | OLR-3 | 8260 | 7050 | 15321 | 7.7 | 7.7 | 7.6 |
| 12/16/2022 | OLR-3 | 9100 | 7285 | 17679 | 7.8 | 7.8 | 7.6 |
| 12/17/2022 | OLR-3 | 8820 | 7050 | 16500 | 7.8 | 7.8 | 7.6 |
| 12/18/2022 | OLR-3 | 8540 | 6815 | 16854 | 7.8 | 7.8 | 7.7 |
| 12/19/2022 | OLR-3 | 8400 | 6580 | 16618 | 7.8 | 7.9 | 7.7 |
| 12/20/2022 | OLR-3 | 8680 | 7050 | 16382 | 7.8 | 7.8 | 7.7 |
| 12/21/2022 | OLR-3 | 8540 | 6815 | 16500 | 7.9 | 7.8 | 7.7 |
| 12/22/2022 | OLR-3 | 8540 | 7285 | 16618 | 7.8 | 7.8 | 7.7 |
| 12/23/2022 | OLR-3 | 8680 | 7050 | 17679 | 7.8 | 7.8 | 7.8 |

### A.2. Batch Digestion Data, Cumulative biogas and methane measured.

**Table A.2.1. Batch Digestion Raw Data – Culbreth.** Batch digestion daily biogas and methane accumulation data for Culbreth duckweed experimental (CDW_1 and CDW_2), inoculum control (CIN_1 and CIN_2) and positive control (CPC_1 and CPC_2) reactors.

| Day | Gas Type | Cumulative Biogas Within Reactor (mL) | | | | | |
|---|---|---|---|---|---|---|---|
| | | CDW_1 | CDW_2 | CIN_1 | CIN_2 | CPC_1 | CPC_2 |
| 1 | Biogas | 354.2775 | 396.4684 | 8.878979 | 51.06987 | 949.4941 | 1236.327 |
| | Methane | 201.0341 | 224.9752 | 0 | 16.47246 | 392.8891 | 511.5769 |
| 2 | Biogas | 575.6853 | 679.7142 | 10.89407 | 114.923 | 1395.053 | 2075.322 |
| | Methane | 326.6714 | 385.7024 | 0.41 | 38.9638 | 577.2561 | 858.7429 |
| 3 | Biogas | 723.6683 | 840.5433 | 43.89112 | 160.7662 | 1715.097 | 2547.294 |
| | Methane | 410.1292 | 476.405 | 0.723 | 48.50678 | 709.686 | 1054.039 |
| 4 | Biogas | 847.4702 | 953.2623 | 87.46739 | 193.2595 | 2059.858 | 2739.25 |
| | Methane | 479.9496 | 539.9749 | 0.9123 | 51.09405 | 852.3441 | 1133.468 |
| 5 | Biogas | 938.1491 | 1049.105 | 117.4418 | 228.3976 | 2280.439 | 2874.207 |
| | Methane | 531.0897 | 594.0272 | 1.115086 | 64.05259 | 989.7269 | 1217.522 |
| 6 | Biogas | 1017.493 | 1146.711 | 134.8849 | 264.1024 | 2325.05 | 2970.863 |
| | Methane | 575.8372 | 649.0738 | 5.35443 | 78.59098 | 1017.511 | 1277.722 |
| 7 | Biogas | 1084.873 | 1228.322 | 145.4641 | 288.9132 | 2444.727 | 3014.743 |
| | Methane | 613.8372 | 695.0999 | 7.593183 | 88.85588 | 1092.049 | 1305.051 |
| 8 | Biogas | 1199.355 | 1292.049 | 204.7833 | 297.4773 | 2508.421 | 3091.495 |
| | Methane | 681.0677 | 732.5241 | 38.31272 | 89.76913 | 1131.719 | 1352.854 |
| 9 | Biogas | 1307.54 | 1344.189 | 251.9489 | 288.5983 | 2559.689 | 3091.495 |
| | Methane | 744.6003 | 763.144 | 63.68962 | 82.23336 | 1169.62 | 1352.854 |
| 10 | Biogas | 1357.035 | 1379.075 | 287.087 | 292.7544 | 2599.739 | 3097.074 |
| | Methane | 773.667 | 783.6312 | 73.31996 | 83.28422 | 1199.228 | 1356.979 |
| 11 | Biogas | 1385.499 | 1412.576 | 309.4419 | 314.1647 | 2629.535 | 3100.392 |
| | Methane | 790.3821 | 803.3049 | 80.64392 | 93.56662 | 2480.313 | 2817.873 |
| 12 | Biogas | 1418.999 | 1447.337 | 323.2956 | 333.0562 | 2647.509 | 3102.503 |
| | Methane | 810.0558 | 823.7181 | 88.78386 | 102.4462 | 1234.544 | 1360.992 |
| 13 | Biogas | 1440.913 | 1477.185 | 335.2602 | 345.7134 | 2654.144 | 3106.876 |
| | Methane | 822.925 | 841.2469 | 90.50356 | 108.8254 | 1239.449 | 1364.225 |
| 14 | Biogas | 1467.487 | 1505.522 | 350.3103 | 361.3304 | 2668.137 | 3123.463 |
| | Methane | 838.5308 | 857.8881 | 96.85231 | 116.2096 | 1249.794 | 2309.106 |
| 15 | Biogas | 1495.069 | 1532.096 | 348.862 | 372.2874 | 2673.928 | 3135.526 |
| | Methane | 858.4274 | 877.0579 | 103.1845 | 121.815 | 1254.074 | 2318.024 |
| 16 | Biogas | 1521.643 | 1550.484 | 359.5042 | 379.1513 | 2686.594 | 3145.327 |
| | Methane | 877.5973 | 890.3224 | 111.8437 | 124.5688 | 1262.692 | 2139.916 |
| 17 | Biogas | 1530.333 | 1561.063 | 367.0608 | 379.5921 | 2698.054 | 3155.279 |
| | Methane | 883.8661 | 897.9539 | 110.8999 | 124.9878 | 1270.489 | 2146.687 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 18 | Biogas | 1549.602 | 1578.443 | 364.416 | 388.3451 | 2718.2 | 3174.43 |
| | Methane | 897.7665 | 910.4916 | 116.6319 | 129.357 | 1284.195 | 2159.716 |
| 19 | Biogas | 1563.33 | 1591.667 | 377.3251 | 395.3979 | 2737.139 | 3182.12 |
| | Methane | 907.6694 | 920.031 | 120.6179 | 132.9796 | 1297.08 | 2164.948 |
| 20 | Biogas | 1566.982 | 1596.831 | 384.6928 | 394.2644 | 2751.977 | 3184.834 |
| | Methane | 910.3041 | 923.756 | 119.0889 | 132.5408 | 1307.175 | 2166.795 |
| 21 | Biogas | 1591.667 | 1623.405 | 390.801 | 409.0627 | 2772.243 | 3174.087 |
| | Methane | 928.1111 | 942.9258 | 125.6225 | 140.4372 | 1320.963 | 2159.483 |
| 22 | Biogas | 1600.861 | 1632.473 | 382.9296 | 416.3045 | 2779.722 | 3180.07 |
| | Methane | 933.4878 | 948.2289 | 129.4637 | 144.2047 | 1326.051 | 2163.553 |
| 23 | Biogas | 1615.093 | 1648.09 | 390.4862 | 423.7981 | 2793.233 | 3193.679 |
| | Methane | 941.8106 | 957.3619 | 132.5071 | 148.0583 | 1700.205 | 1943.952 |
| 24 | Biogas | 1616.478 | 1655.394 | 391.7456 | 421.8459 | 2797.214 | 3193.679 |
| | Methane | 942.6208 | 961.6338 | 128.2856 | 147.2986 | 1702.628 | 1943.952 |
| 25 | Biogas | 1632.095 | 1674.286 | 391.6196 | 432.677 | 2808.674 | 3201.905 |
| | Methane | 951.7538 | 972.6817 | 131.99 | 152.9179 | 1709.604 | 1948.959 |
| 26 | Biogas | 1634.866 | 1688.391 | 390.6751 | 445.2713 | 2808.674 | 3202.354 |
| | Methane | 953.3742 | 980.9309 | 132.188 | 159.7447 | 1709.604 | 1949.232 |
| 27 | Biogas | 1634.362 | 1688.895 | 390.6751 | 446.1529 | 2808.674 | 3229.317 |
| | Methane | 953.0796 | 981.2255 | 132.085 | 160.2309 | 1709.604 | 1965.644 |
| 28 | Biogas | 1633.858 | 1690.28 | 390.674 | 447.0975 | 2778.873 | 3228.714 |
| | Methane | 952.785 | 982.0357 | 131.5326 | 160.7833 | 1691.464 | 1965.277 |

**Table A.2.2. Batch Digestion Raw Data – Raleigh.** Batch digestion daily biogas and methane accumulation data for Raleigh duckweed experimental (RDW_1 and RDW_2), inoculum control (RIN_1 and RIN_2) and positive control (RPC_1 and RPC_2) reactors.

| Day | Cumulative Volume (mL) | Cumulative Gas Produced by Each Reactor (mL) | | | | | |
|---|---|---|---|---|---|---|---|
| | | RDW_1 | RDW_2 | RIN_1 | RIN_2 | RPC_1 | RPC_2 |
| 1 | Biogas | 452.3871 | 448.7348 | 8.753036 | 20.90653 | 991.297 | 1072.606 |
| | Methane | 213.7516 | 212.0259 | 0.89652 | 2.697946 | 464.394 | 502.4851 |
| 2 | Biogas | 796.9671 | 864.3465 | 21.53625 | 76.13252 | 1492.676 | 1573.353 |
| | Methane | 376.5647 | 408.4013 | 1.78902 | 22.56646 | 699.2756 | 737.0704 |
| 3 | Biogas | 1059.684 | 1138.902 | 24.55888 | 100.7544 | 1835.115 | 1927.554 |
| | Methane | 500.6978 | 538.1281 | 1.90762 | 28.29286 | 859.6983 | 903.0036 |
| 4 | Biogas | 1248.85 | 1338.774 | 32.2414 | 122.1647 | 2204.002 | 2367.88 |
| | Methane | 590.0783 | 632.5668 | 1.90762 | 33.33718 | 1032.511 | 1109.283 |
| 5 | Biogas | 1397.841 | 1454.641 | 60.26371 | 117.064 | 2440.019 | 2440.599 |
| | Methane | 674.4572 | 698.1869 | 3.465527 | 27.19523 | 1192.511 | 1158.581 |
| 6 | Biogas | 1445.447 | 1508.293 | 78.08464 | 140.9302 | 2487.751 | 2581.328 |
| | Methane | 701.4185 | 728.5719 | 7.641572 | 34.79493 | 1224.869 | 1253.983 |
| 7 | Biogas | 1500.988 | 1563.708 | 102.9584 | 165.678 | 2552.36 | 2677.978 |
| | Methane | 732.8734 | 759.9554 | 14.68576 | 41.7678 | 1268.668 | 1319.503 |
| 8 | Biogas | 1587.637 | 1646.704 | 122.1647 | 181.2319 | 2618.858 | 2742.083 |
| | Methane | 781.9458 | 806.9594 | 20.62952 | 45.64309 | 1313.748 | 1362.961 |
| 9 | Biogas | 1646.83 | 1705.897 | 137.7186 | 196.7859 | 2672.384 | 2742.083 |
| | Methane | 820.4155 | 845.4291 | 27.00892 | 52.02249 | 1348.649 | 1362.961 |
| 10 | Biogas | 1677.56 | 1736.502 | 150.4389 | 209.3802 | 2714.197 | 2746.742 |
| | Methane | 840.387 | 865.3187 | 32.24118 | 57.1729 | 1375.913 | 1365.999 |
| 11 | Biogas | 1707.787 | 1766.854 | 160.4513 | 219.5186 | 2745.305 | 2749.513 |
| | Methane | 860.0311 | 885.0446 | 36.33265 | 61.34623 | 2749.513 | 2557.424 |
| 12 | Biogas | 1727.308 | 1788.768 | 165.615 | 227.0752 | 2764.07 | 2751.276 |
| | Methane | 872.7179 | 899.2866 | 38.16366 | 64.73239 | 1408.433 | 1368.956 |
| 13 | Biogas | 1737.761 | 1799.347 | 167.882 | 229.4681 | 2770.997 | 2754.929 |
| | Methane | 879.5114 | 906.162 | 39.07835 | 65.72893 | 1412.95 | 1371.337 |
| 14 | Biogas | 1757.03 | 1818.616 | 180.2873 | 241.8735 | 2785.606 | 2768.782 |
| | Methane | 892.0345 | 918.6851 | 44.16637 | 70.81695 | 1422.476 | 1380.37 |
| 15 | Biogas | 1773.025 | 1834.611 | 186.0178 | 247.6039 | 2791.652 | 2784.782 |
| | Methane | 902.4295 | 929.0801 | 46.51668 | 73.16726 | 1426.417 | 1390.803 |
| 16 | Biogas | 1781.337 | 1842.923 | 191.9371 | 253.5232 | 2804.876 | 2795.444 |
| | Methane | 907.4335 | 934.0841 | 50.32115 | 76.97173 | 1435.505 | 1398.13 |
| 17 | Biogas | 1779.952 | 1841.538 | 193.7632 | 255.3494 | 2816.84 | 2823.044 |
| | Methane | 906.5995 | 933.2501 | 51.49487 | 78.14545 | 1443.728 | 1417.098 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 18 | Biogas | 1794.183 | 1855.77 | 204.3425 | 265.9286 | 2837.873 | 2883.351 |
| | Methane | 915.1669 | 941.8175 | 58.29435 | 84.94494 | 1458.182 | 1458.543 |
| 19 | Biogas | 1807.407 | 1868.994 | 205.2241 | 266.8102 | 2857.646 | 2885.774 |
| | Methane | 923.1278 | 949.7784 | 58.86098 | 85.51156 | 1471.771 | 1460.208 |
| 20 | Biogas | 1807.03 | 1868.49 | 206.9243 | 268.3845 | 2873.137 | 2892.041 |
| | Methane | 922.9003 | 949.4751 | 59.95119 | 86.52595 | 1482.417 | 1464.515 |
| 21 | Biogas | 1831.714 | 1893.301 | 220.5891 | 282.1752 | 2894.295 | 2896.887 |
| | Methane | 937.7606 | 964.4112 | 68.73642 | 95.387 | 1496.958 | 1467.846 |
| 22 | Biogas | 1844.057 | 1905.643 | 225.8787 | 287.4648 | 2918.104 | 2901.925 |
| | Methane | 945.1907 | 971.8413 | 72.13616 | 98.78674 | 1513.32 | 1471.308 |
| 23 | Biogas | 1852.873 | 1914.333 | 237.1506 | 298.6108 | 2928.209 | 2909.386 |
| | Methane | 951.0592 | 977.626 | 77.91911 | 104.4859 | 1520.035 | 1476.266 |
| 24 | Biogas | 1849.598 | 1911.184 | 240.4881 | 302.0742 | 2932.365 | 2909.386 |
| | Methane | 948.8795 | 975.5301 | 79.61886 | 106.2694 | 1522.797 | 1476.266 |
| 25 | Biogas | 1860.555 | 1922.141 | 251.0673 | 312.6534 | 2936.33 | 2912.313 |
| | Methane | 956.1732 | 982.8238 | 85.03737 | 111.6879 | 1525.432 | 1478.211 |
| 26 | Biogas | 1858.792 | 1920.378 | 253.1454 | 314.7315 | 2940.33 | 2919.531 |
| | Methane | 954.9995 | 981.6501 | 86.10171 | 112.7523 | 1528.09 | 1483.007 |
| 27 | Biogas | 1858.792 | 1920.378 | 253.3972 | 314.9833 | 2940.33 | 2919.891 |
| | Methane | 954.9995 | 981.6501 | 86.23073 | 112.8813 | 1528.09 | 1483.246 |
| 28 | Biogas | 1855.518 | 1917.104 | 253.3343 | 314.9204 | 2944.4 | 2919.891 |
| | Methane | 952.8198 | 979.4703 | 86.19847 | 112.8491 | 1530.794 | 1483.246 |

**Table A.2.3. Batch Digestion Raw Data – Sicily.** Batch digestion daily biogas and methane accumulation data for Sicily duckweed experimental (SDW_1 and SDW_2), inoculum control (SIN_1 and SIN_2) and positive control (SPC_1 and SPC_2) reactors.

| Day | Cumulative Volume (mL) | Cumulative Gas Produced Per Reactor (mL) | | | | | |
|---|---|---|---|---|---|---|---|
| | | SDW_1 | SDW_2 | SIN_1 | SIN_2 | SPC_1 | SPC_2 |
| 1 | Biogas | 464.7295 | 468.3819 | 18.13579 | 21.78813 | 196.2821 | 209.4431 |
| | Methane | 224.6312 | 226.3966 | 2.368106 | 0.994237 | 79.09615 | 84.39968 |
| 2 | Biogas | 929.4591 | 913.8421 | 37.0902 | 21.47327 | 331.5448 | 335.4806 |
| | Methane | 449.2624 | 441.7138 | 8.542819 | 4.1335 | 133.6032 | 135.1892 |
| 3 | Biogas | 1152.63 | 1171.018 | 33.68974 | 52.07741 | 372.3504 | 375.5934 |
| | Methane | 557.1341 | 566.0219 | 2.539663 | 11.42751 | 150.0467 | 151.3535 |
| 4 | Biogas | 1288.774 | 1314.467 | 43.26141 | 68.95377 | 418.6029 | 418.0991 |
| | Methane | 622.9407 | 635.3593 | 2.927798 | 15.34643 | 168.6852 | 168.4822 |
| 5 | Biogas | 1414.843 | 1444.566 | 51.13284 | 80.85538 | 459.6288 | 459.4714 |
| | Methane | 703.7044 | 718.7049 | 7.458151 | 22.45864 | 196.3034 | 196.3335 |
| 6 | Biogas | 1503.759 | 1558.292 | 42.37981 | 96.91311 | 514.7604 | 513.6584 |
| | Methane | 760.6666 | 791.5616 | 7.458151 | 31.23377 | 233.4174 | 232.8116 |
| 7 | Biogas | 1558.67 | 1643.556 | 35.45294 | 120.3385 | 569.64 | 568.7584 |
| | Methane | 795.8444 | 846.184 | 7.458151 | 42.82587 | 270.3618 | 269.9044 |
| 8 | Biogas | 1612.322 | 1713.58 | 38.28666 | 139.5448 | 626.5347 | 622.0323 |
| | Methane | 830.2153 | 891.0438 | 13.43088 | 50.56809 | 308.6627 | 305.7679 |
| 9 | Biogas | 1682.472 | 1787.383 | 45.40244 | 150.3129 | 658.9651 | 654.5885 |
| | Methane | 875.4996 | 938.6858 | 17.27669 | 62.87351 | 331.8008 | 328.9958 |
| 10 | Biogas | 1747.081 | 1849.724 | 62.78257 | 165.4261 | 698.7315 | 694.0087 |
| | Methane | 917.2067 | 978.9294 | 23.44336 | 67.33508 | 360.173 | 357.1208 |
| 11 | Biogas | 1816.349 | 1909.799 | 82.55561 | 176.0053 | 722.5663 | 717.497 |
| | Methane | 961.9219 | 1017.71 | 25.9004 | 69.2187 | 717.497 | 687.7115 |
| 12 | Biogas | 1868.994 | 1962.065 | 94.39425 | 187.4661 | 735.8532 | 730.5636 |
| | Methane | 995.9054 | 1051.449 | 28.60631 | 72.82062 | 386.6582 | 383.2017 |
| 13 | Biogas | 1891.034 | 1968.866 | 105.918 | 183.7508 | 744.7637 | 739.2537 |
| | Methane | 1010.133 | 1055.84 | 30.88492 | 69.14994 | 393.0155 | 389.4018 |
| 14 | Biogas | 1912.066 | 1995.944 | 116.6862 | 200.5642 | 750.5886 | 744.9841 |
| | Methane | 1023.71 | 1073.319 | 34.57678 | 75.5094 | 397.1714 | 393.4902 |
| 15 | Biogas | 1926.298 | 2007.405 | 123.739 | 204.8462 | 753.4538 | 747.7548 |
| | Methane | 1032.897 | 1080.717 | 61.83671 | 76.4267 | 399.2819 | 395.5312 |
| 16 | Biogas | 1937.255 | 2025.793 | 127.3913 | 215.9292 | 755.6893 | 749.4551 |
| | Methane | 1039.982 | 1092.607 | 62.17424 | 83.51005 | 400.9286 | 396.7836 |
| 17 | Biogas | 1952.872 | 2043.676 | 127.2024 | 218.0073 | 757.5154 | 751.1868 |
| | Methane | 1050.08 | 1104.171 | 62.72118 | 88.66776 | 402.2738 | 398.0592 |
| 18 | Biogas | 1999.219 | 2072.014 | 148.2978 | 221.0929 | 764.8516 | 758.6174 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Methane | 1080.049 | 1122.494 | 67.47693 | 104.2823 | 407.6777 | 403.5328 |
| 19 | Biogas | 2009.672 | 2089.394 | 150.5648 | 230.2867 | 771.621 | 765.3869 |
| | Methane | 1086.808 | 1133.732 | 68.09995 | 109.0988 | 412.6642 | 408.5192 |
| 20 | Biogas | 2021.133 | 2100.477 | 151.6983 | 231.0424 | 777.4774 | 771.2432 |
| | Methane | 1094.219 | 1140.899 | 79.64691 | 109.4015 | 416.9781 | 412.8331 |
| 21 | Biogas | 2039.52 | 2119.746 | 162.4664 | 242.6921 | 785.034 | 778.7998 |
| | Methane | 1106.108 | 1153.359 | 78.6665 | 114.7273 | 422.5444 | 418.3994 |
| 22 | Biogas | 2045.943 | 2126.169 | 163.8518 | 244.0775 | 788.9067 | 782.6725 |
| | Methane | 1110.262 | 1157.512 | 82.0728 | 115.3503 | 425.4129 | 421.2679 |
| 23 | Biogas | 2059.671 | 2127.932 | 178.0204 | 246.2815 | 793.3777 | 786.9231 |
| | Methane | 1119.397 | 1158.685 | 82.06497 | 118.9348 | 428.7244 | 424.4162 |
| 24 | Biogas | 2061.56 | 2130.199 | 176.0053 | 244.6442 | 793.6296 | 787.3954 |
| | Methane | 1120.655 | 1160.194 | 82.75202 | 118.2058 | 428.911 | 424.766 |
| 25 | Biogas | 2073.399 | 2142.164 | 183.31 | 252.0748 | 796.5892 | 790.2606 |
| | Methane | 1128.533 | 1168.156 | 82.37808 | 121.6959 | 431.1032 | 426.8882 |
| 26 | Biogas | 2073.903 | 2143.045 | 183.373 | 252.5156 | 798.1005 | 791.7404 |
| | Methane | 1128.869 | 1168.743 | 83.11156 | 121.9395 | 432.2226 | 427.9843 |
| 27 | Biogas | 2073.399 | 2143.045 | 184.9472 | 254.5937 | 798.1005 | 792.7795 |
| | Methane | 1128.533 | 1168.743 | 83.11278 | 122.9618 | 432.2226 | 428.7539 |
| 28 | Biogas | 2073.903 | 2144.053 | 184.2546 | 254.4048 | 798.4469 | 792.8739 |
| | Methane | 1128.869 | 1169.414 | 83.89776 | 122.9232 | 432.4791 | 428.8239 |