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Kinetic Modeling of Thermophilic Anaerobic Digestion of Lemnaceae for Biogas Production

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Abstract
Anaerobic digestion of sustainably sourced biomass to generate biogas is a vital form of renewable energy that provides significant benefits to the environment. Lemnaceae, commonly referred to as duckweed, has shown great potential as a next-generation biomass feedstock for anaerobic digestion due to its rapid growth rates, low lignin content, and ability to remove nutrients from wastewater. However, research in this area is largely focused on the mesophilic (35 °C) anaerobic digestion of duckweed. For the first time, batch thermophilic anaerobic digestion was performed using three different duckweed varieties grown on swine lagoon wastewater to ascertain the biochemical methane potential (BMP) of the biomasses and estimate parameters associated with the kinetics of the digestion process. The BMPs of the three duckweed varieties were 205 ± 5, 217 ± 5, and 262 ± 7 mL CH_4 g^{-1} volatile solids (VS) for the local variety (OxNC), *Lemna gibba* (8678), and *Lemna gibba* (7741), respectively. Four kinetic models were fitted to the experimental data: first order, modified Gompertz, transference, and logistic function. Unique to this study, inoculum from continuous thermophilic anaerobic digesters processing identical feedstocks was used during the BMP, causing the absence of a lag phase. The first-order model predicted the hydrolysis constant (k) to be 0.205–0.285 day^{-1}, which is similar to the hydrolysis constants reported in the literature for effective anaerobic digestion systems, thereby demonstrating that duckweed biomass has viable degradation rates. In this work, BMP experimentation and kinetic modeling have demonstrated the viability of anaerobically digesting multiple varieties of duckweed biomass under thermophilic conditions.

Keywords Duckweed · Anaerobic digestion · Biochemical methane potential

Introduction

The Lemnaceae family, often referred to as duckweeds, are the fastest growing higher plants in the world and have been well-researched as a feedstock for biogas production because of their composition and ability to be grown decoupled from arable land [1]. Duckweeds rapidly draw up nutrients from their growth medium while they photosynthesize, producing a plant that is high in both starch and proteins [2]. Anaerobic digestion is a well-developed technology that effectively converts waste streams, agricultural residues, and energy crops into renewable natural gas (bio-methane) [3, 4]. Including biogas, which can be used for heat and electricity, a nitrogen- and phosphorus-rich digestate is produced that can be used as a bio-organic fertilizer [3, 5–10]. This study investigates the biochemical methane potential of three duckweed varieties grown on swine wastewater under thermophilic (50 °C) anaerobic digestion conditions and uses kinetic modeling to determine key reaction parameters.

The anaerobic digestion of duckweeds has been investigated with many studies evaluating the plants as co-feedstocks combined with animal manure, food waste, and wastewater for mesophilic (35 °C) anaerobic digestion with and without pretreatments [11–16]. All co-digestion studies came to the conclusion that the addition of duckweed to the anaerobic digestion of the primary substrate increased methane yields. This phenomenon was often attributed to the increase in microbial diversity after the addition of a more complex mixed substrate but could also be attributed to the plant's customizable C/N content [2, 13, 15, 17]. The composition of some

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duckweeds can be designed by altering the nutrient loading of the growth medium, and effectively starving the plant prior to harvesting it. Nutrient starvation results in an increased starch content and decreased protien content, or a higher C/N ratio [17]. Duckweeds are a promising feedstock for anaerobic digestion because of their rapid growth rates, constant harvesting potential, and ability to be enigneered to compliment specific biogas production systems.

Primary knowledge gaps in the field of duckweed-based biogas production remain in the anaerobic digestion of the plant under thermophilic (50 °C) conditions. Although thermophilic systems can be more sensitive to perturbations, they enable the operator to conduct continuous digestion with a shorter hydraulic retention time, thereby enabling higher throughput or smaller reactor size [18]. The effluent from thermophilic anaerobic digesters can be categorized as a class A fertilizer, because the higher operating temperature can remove pathogens contained in the substrate [19]. The few existing studies evaluating thermophilic anaerobic digestion of duckweed have focused on the impact of duckweed growth mediums and acidogenic digestion for the recovery of carboxylic acid [20, 21]. A biochemical methane potential (BMP) assay of duckweeds as single-substrate for thermophilic anaerobic digestion has not been conducted and the reaction kinetics of such a system have not been investigated. For the first time, a comprehensive evaluation of duckweed as a feedstock for thermophilic anaerobic digestion is completed using rigorous experimental and modeling techniques.

Batch anaerobic digestion tests are typically conducted for two primary goals: first to determine the ultimate methane yield from a substrate (BMP) and second to model the kinetic processes within the reaction and specify governing parameters of kinetic equations. Three duckweed varieties were selected and grown for this BMP assay. The first was *Lemna gibba* (Rutgers Duckweed Stock Cooperative (RDSC) accession 8678), selected based on the findings of a previous in vitro study in which 41 duckweed clones were evaluated for growth on swine lagoon effluent [22]. The second was another *Lemna gibba* (RDSC accession 7741), selected based on past performance in an outdoor cultivation system. The third duckweed variety chosen for this study was found locally in Oxford, NC, USA, has not been genotyped, and is referred to as OxNC. To the knowledge of the authors, these duckweed varieties have not been evaluated for biochemical methane potential or kinetic parameterization.

Mathematical modeling of biochemical reactions is a critical step in understanding a particular anaerobic digestion system because it enables the determination of parameters necessary for the optimization, simulation, and scaling-up of a system, and also because kinetic modeling can be used as a method of validating experimental data [12, 23–25]. Previously, studies have conducted mesophilic batch anaerobic digestion using duckweed as a co-feedstock and conducted kinetic modeling using the modified Gompertz equation [12, 13]. For a complete kinetic evaluation of thermophilic duckweed-based anaerobic digestion, it is necessary for multiple kinetic models to be applied and evaluated. This study evaluated the fits of four popular kinetic models selected from previous work in this area: the first-order model, the modified Gompertz equation, the transference model, and the logistic function [25–29]. These models predict kinetic parameters including ultimate methane yields, hydrolysis constants, lag times, and maximum methane production rates. An understanding of the reaction kinetics of the thermophilic anaerobic digestion of duckweed is vital to downstream applications.

In parallel to the batch digestion described herein, we conducted continuous thermophilic anaerobic digestion so that steady-state culture from those reactors could serve as acclimated inocula for the batch reactors in this study. The conditions of the continuous anaerobic digestion are described briefly in this study, but more thoroughly elsewhere [30]. The kinetic modeling conducted in this study could help in the parameterization and engineering of future large-scale thermophilic anaerobic digestion of duckweed. For the first time, this study investigated batch thermophilic anaerobic digestion of duckweed as a single substrate to elucidate biochemical methane potentials for three duckweed varieties and the complete results of the experiment were used to fit four kinetic models to investigate the suitability of the substrates.

Methods and Materials

Substrate and Inoculum

Three duckweeds were prepared under identical conditions for use in this study. Two clones, 8678 (Srinagar, Kashmir, India) and 7741 (Syracuse, Sicilia, Italy), were selected based on their growth in synthetic and diluted swine wastewater as determined by a previous study [22]. 8678 and 7741 are *Lemna gibba* clones acquired from the RDSC. OxNC is a wild, locally adapted variety collected from an agricultural pond in Oxford NC. 3.78 L of live duckweed was collected and the OxNC culture was started directly from this sample. OxNC appears to be a monoculture with all fronds looking identical. However, since the OxNC culture was not started from an individual frond, it may contain some genetic diversity among individual and we refer to the three different duckweed genotypes (8678, 7741 and OxNC) as varieties.

Table 1 Biomass characteristics of *Lemna gibba* (7741), local variant (OxNC), and *Lemna gibba* (8678)

Parameter	7741	OxNC	8678
Total Kjeldahl nitrogen (g/L)	43.2 ± 0.8	47.7 ± 0.3	44.2 ± 1.1
Chemical oxygen demand (g/kg)	898.5 ± 9.1	913.7 ± 2.3	916.1 ± 4.9
Total phosphorus (g/L)	26.5 ± 1.1	14.7 ± 0.7	23.5 ± 1.6
Total solids (%)	88.52 ± 0.27	96.79 ± 0.37	89.53 ± 0.40
Volatile solids (%)	74.84 ± 0.07	84.39 ± 0.5	75.89 ± 0.06

Duckweed was cultivated in a 27-m^2 hydroponic system inside a greenhouse on the NCSU Lake Wheeler Road Field Laboratory. The water depth was 0.45 m and the system was divided into six sections with each section having 4 m^2 of growing area (the remaining 3 m^2 are covered by a walkway that goes down the center of the system to provide access). Duckweed cultures were grown in duplicate with two cultures for each of the three varieties. Duckweed cultures were continuously cultivated from November 2021 through May 2022. The system started off with a 1:10 dilution of swine lagoon top water to give a starting concentration of 15 mg/L total N and 5 mg/L ortho-phosphate. Twice per week, the system was topped off with lagoon topwater to replace any water lost through evaporation or harvesting. On average, ~ 400 L of topwater was added to the system every week. During this period, a linear increase in N and P was observed with max concentrations of 46 mg/L total N and 21 mg/L ortho-phosphate. Biomass was harvested two times per week. At each harvest, 10% of each culture was collected and dried in mesh bags in the sun. The Lemnaceae or duckweed biomass characteristics are shown in Table 1. Dried duckweed biomass was milled using an IKA MF 10.1 biomass mill with a 0.25 sieve (0.25 mm hole size) and stored in plastic bags at room temperature.

The Lemnaceae or duckweed biomass from each variety was anaerobically digested in separate continuously stirred tank reactors (CSTRs) under thermophilic conditions (50 °C) for biogas production. The hydraulic retention time (HRT) in each CSTR was 10 days and the organic loading rate was 2000 mg COD L^{-1} digester day^{-1}. The three CSTRs were operated at steady state for over 150 days with consistent removal of chemical oxygen demand (COD), daily biogas production rate, and methane content in the biogas [30]. The culture from each CSTR at steady state was then taken as the inoculum for the batch digestion of the duckweed biomass from the respective variety under the thermophilic condition of 50 °C.

Biochemical Methane Potential Analysis

The standard BMP test procedure outlined by Holliger et al. was used for this study [31]. Briefly, ANKOM bioreactors (RF Gas Production System, Macedon, NY), equipped with automatic pressure sensing modules, were used for the BMP test with 400 mL working volumes and 622 mL total volumes. ANKOM modules wirelessly monitor the difference between the external (atmospheric) pressure and internal pressure within the batch reactor. This difference is recorded digitally at user-defined intervals (every 10 min in this experiment) and the built-up pressure is released and measured by the module once it meets a safety threshold. This pressure data is converted to volumetric quantities via the ideal gas law (Eq. 1) and coupled with frequent gas compositional analyses outlined in the "Analytical Methods" section. The three duckweed varieties, their associated inoculum controls, and pure corn starch (positive control) reactors were tested in duplicate. A total volatile solids (VS) content of 20 g VS/L with an initial substrate VS to inoculum VS ratio of 1:1 (4 g VS from both inoculum and substrate) was used. After the reactor contents were prepared, the media and headspace were purged using nitrogen gas (99.99%) to ensure an absence of oxygen. The reactors were incubated at 50 °C in temperature-controlled water baths with constant magnetic stirring. Headspace pressure was set to 3.3 psi as recommended by Yan et al. [32]. The length of the batch digestion was not predetermined; alternatively, the BMP test continued until biogas production for 3 consecutive days was less than 1% of the total biogas produced, which for this experiment was 28 days. A schematic of the BMP assay is shown in Fig. 1.

Biochemical methane potential for the experimental and control assays was calculated under standard conditions and expressed as volume of methane per unit mass as volatile solids from the substrate (N mL CH_4 g^{-1} VS). 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) was utilized:

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

where n = gas produced in moles; p = pressure in kilopascal; V = volume of reactor headspace in liters; T = temperature in kelvin; and R = gas constant (8.314 L kPa $K^{-1}mol^{-1}$). Avogadro's law was then used to convert moles of biogas to milliliters. Biochemical methane volumes were then determined using results from the biogas composition analyses in the "Analytical Methods" section.

To validate the three inocula used for this experiment, starch was chosen as the positive control. The theoretical

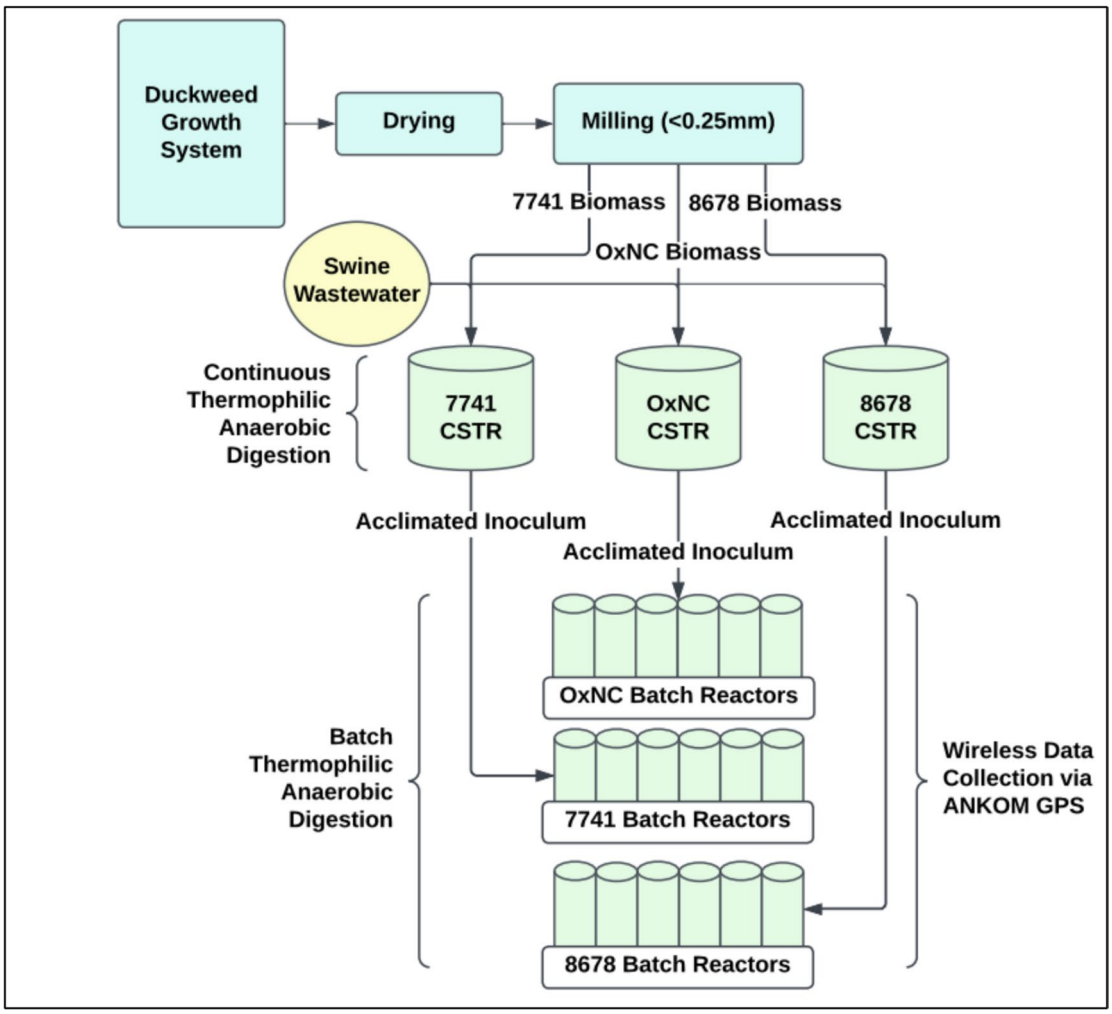


Fig. 1 Schematic of BMP assay for three duckweed varieties including the identical growth, drying, and milling treatments; continuous co-digestion with swine wastewater; and batch digestion using duckweed biomass and acclimated inoculum from the three continuously stirred tank reactors (CSTRs)

biochemical methane potential of corn starch was calculated using a procedure outlined by several studies (Eq. 2) [33–35]. Briefly, the theoretical biochemical methane potential (TBMP) of a compound or feedstock can be calculated using the elemental composition of the biomass and the Buswell equation (Eq. 2):

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

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 $(C_6H_{10}O_5)_n$ [36]. Using the molar ratios of carbon, hydrogen, and oxygen of starch, the TBMP can be calculated to be 414.4 mL CH_4 g^{-1}VS.

Analytical Methods

Total chemical oxygen demand (TCOD), total solids (TS), and VS analyses of the three inocula, three duckweed biomass samples, and corn starch samples were conducted by the North Carolina State University Environmental Analysis

Lab according to APHA Standard Methods [37]. Biogas production was measured constantly using the ANKOM modules. Biogas composition samples were taken after 3, 6, 12, 18, 24, and 28 days of the test. 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, PA) and with helium as the carrier gas.

Kinetic Modeling and Model Selection

Four models were selected to estimate anaerobic digestion performance parameters from the data collected in the BMP experiment: first-order, modified Gompertz equation, transference, and logistic function models. The first-order kinetic model (Eq. 3) is a conventional method developed from microbial growth kinetics 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 [25, 26, 38]. The first-order model assumes a constant rate constant (k) and a single-reactant system with no intermediates. It has been used successfully and deemed appropriate for anaerobic digestion systems processing animal manures, okra waste, and granular feedstocks, such as bagasse [26–28].

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

where $M(t)$ is the cumulative methane (mL g^{-1}VS) (represented by net methane) at digestion time t (day), M_u is the ultimate methane yield (mL g^{-1}VS), and k is the hydrolysis constant or first-order rate constant (day^{-1}). Unlike the other models employed in this study, the first-order model does not account for lag time as a variable. The modified Gompertz equation (Eq. 4) is a common kinetic model selected for BMP estimation and includes lag time, or time until biogas production, as a variable. The modified Gompertz equation was originally designed to predict human mortality rates and is frequently applied to microbial growth-dependent processes, including anaerobic digestion [39]. The primary assumption of the model is that growth is related to mortality, and that the latter increases exponentially with time. The modified Gompertz equation has been found to fit well to data resulting from the mesophilic anaerobic digestion of food waste, food waste co-digested with duckweed, waste activated sludge co-digested with duckweed, and systems with significant lag phases [11–13, 24, 29].

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

where R_m is the maximum methane production rate (mL g^{-1}VSday^{-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 microbial growth rates [27, 29, 40]. Alqaralleh et al. found that the transference model, also called the reaction curve model, supported the best fit to their thermophilic anaerobic digestion data obtained using waste activated sludge and fat, oil, and grease (FOG) co-feedstocks when compared to the modified Gompertz equation and first-order models [29].

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

where $M(t)$, M_u, t, R_m, and λ have the same definitions as previously reported. The transference model, when the experimental system does not have a lag period, simplifies to Eq. 6.

$$M(t) = M_u\left(1 - \exp\left(-\frac{R_m \times t}{M_u}\right)\right) \tag{6}$$

The implications of this simplification are discussed in the Results section. The logistic function model (Eq. 7) also assumes governing microbial growth kinetics and predicts the same parameters as the transference and modified Gompertz equations [23, 27]. The logistic function model assumes that the rate of methane production is proportional to the accumulated biogas volume and that the rate slows down as it approaches the maximum methane yield [39]. The logistic function has shown accurate prediction and fit to experimental data that show short lag phases [24, 25]. The logistic function model has outperformed the first-order model for the mesophilic anaerobic digestion of cattle manure [25].

$$M(t) = \frac{M_u}{1 + \exp\left[\frac{4 \times R_m}{Mu} \times (\lambda - t) + 2\right]} \tag{7}$$

where $M(t)$, M_u, t, R_m, and λ have the same definitions as previously reported.

Goodness of fit for the chosen models can be quickly judged by R^2 (Eq. 8) and adjusted-R^2 (Eq. 9) values. However, there are several other criteria that can determine the best model for predicting an experimental data set [23]. This study also included the residual sum of squares (RSS, Eq. 10) also known as the sum of squared estimate of errors (SSE), residual standard error (RSE, Eq. 11), and root mean square error (RMSE, Eq. 12) for goodness of fit [41, 42]. This study also calculated the corrected Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) which are two criteria used to reduce overfitting in the model, where lower values indicate a better fitting to the model [23, 43]. AIC and BIC formulas are detailed in Eq. 13 and Eq. 14, respectively:

$$R^2 = 1 - \frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{N}(y_i - \bar{y})^2} \tag{8}$$

$$\text{Adjusted } R^2 = 1 - \frac{(1-R^2)(N-1)}{N-p-1} \tag{9}$$

$$\text{RSS} = \text{SSE} = \sum_{i=1}^{N} (y_i - \hat{y}_i)^2 \tag{10}$$

$$\text{RSE} = \sqrt{\frac{1}{N-2}\text{RSS}} \tag{11}$$

$$\text{RMSE} = \sqrt{\frac{1}{N}\text{RSS}} \tag{12}$$

$$\text{AIC} = N \times \ln\left(\frac{\text{RSS}}{N}\right) + 2K + \frac{2K(K+1)}{N-K-1} \tag{13}$$

$$\text{BIC} = \ln\left(\frac{\text{RSS}}{N}\right) + K \times \ln(N) \tag{14}$$

where y_i is the experimental value, $\hat{y}_i$ is the predicted value, $\bar{y}$ is the mean value, N is the number of data points, p is the number of independent variables, RSS is the residual sum of squares, and K is the number of parameters the model is used to fit plus one [43]. 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.

Statistics and Data Processing

Biochemical methane potential results were recorded by the automated ANKOM software, and Microsoft Excel software was used to determine the statistical significance of these raw results via *t*-tests (p-value = 0.05) performed in matrix between each duckweed variety.

Results and Discussion

Biochemical Methane Potential Assay

Following standard BMP test procedures, methane production results are described as net mL CH_4 g^{-1}VS. Net biogas and net methane are used as the measurements for biochemical methane 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 ± 3.9, 351.9 ± 3.7, and 405.0 ± 2.6 mL CH_4 g^{-1}VS for the OxNC, 8678, and 7741 inocula, respectively, which correspond to 90%, 85%, and 97% of the theoretical biochemical methane 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 batch digestion [31].

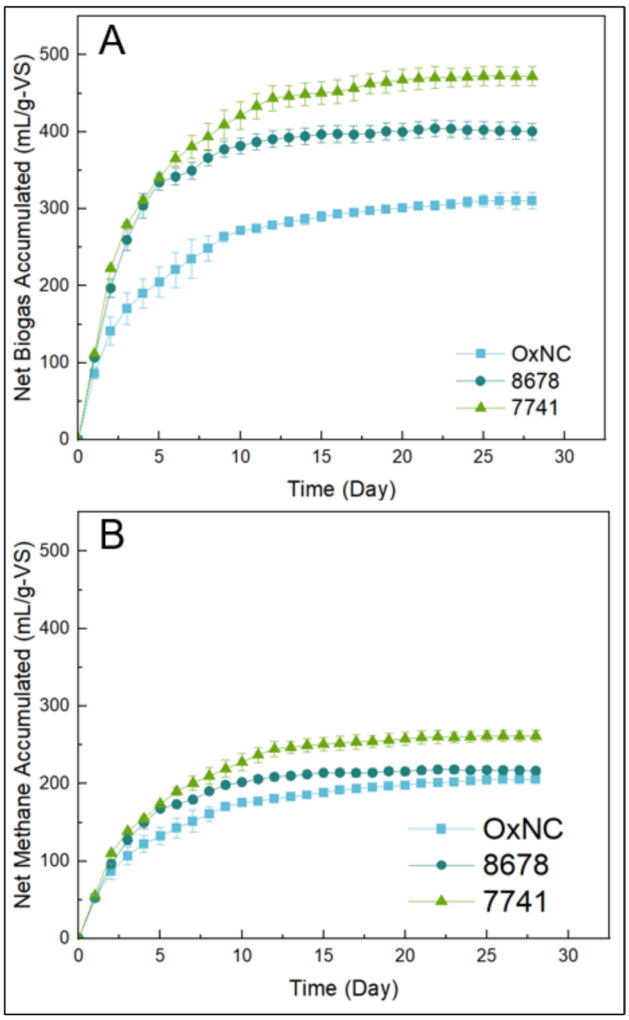


Fig. 2 Biogas and methane production curves for biochemical methane potential (BMP) assay. Net biogas (**A**) and methane (**B**) accumulated (mL $g^{-1}VS_{fed}$) for each duckweed variety during the 28-day batch test

Figure 2 displays the biogas and methane production curves (mL $g^{-1}VS_{fed}$) as volume accumulated throughout the 28-day batch test. SI Table 1 in the Supplementary Information presents the complete summary of results from the BMP batch test. The final methane production rates, or BMPs, for the three duckweed varieties were 205 ± 5, 217 ± 5, and 262 ± 7 mL CH_4 $g^{-1}VS_{fed}$ for the OxNC, 8678, and 7741 duckweed varieties, respectively. *T*-tests resulted in *p*-values that support the conclusion that there was a significant ($p = 0.0084$, $p = 0.0169$) difference between the methane production of the 7741 duckweed compared to the OxNC and 8678 varieties, respectively (which did not show significant difference ($p = 0.2100$)). A complete table of *p*-values

is listed in SI Table 2. The 7741 duckweed produced significantly more methane per unit mass VS fed than the other two varieties. One possible reason for the 7741 reactors to outperform the OxNC and 8678 varieties is the fact that the 7741 duckweed has the highest loading rate in terms of chemical oxygen demand (g TCOD/L). By using an organic loading rate based on volatile solids, the TCOD-based loading rates of each variety varied and were 11.0, 12.6, and 13.5 g TCOD/L for the OxNC, 8678, and 7741 varieties, respectively. It is consistent with the literature in this field that a higher loading rate (in terms of TCOD) for the 7741 reactors would result in a higher BMP result [44]. The highest BMP result from this study of 262 ± 7 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 studies evaluating the BMP of duckweeds at mesophilic conditions [45, 46]. This result is also comparable to those produced by experiments conducting mesophilic anaerobic digestion of duckweed that had undergone 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) [13, 46]. The BMP determined in this batch experiment was lower than those obtained during the co-digestion of duckweeds with concentrated municipal and swine wastewaters (340 and 361 mL CH_4 g^{-1}VS, respectively) and thermally pretreated waste activated sludge (468 mL CH_4 g^{-1}VS) [11, 16]. This supports previous conclusions that duckweeds often perform better as co-substrates (depending on the composition of the secondary feedstock) for anaerobic digestion, and that there are available pretreatment strategies to enhance existing methane generation [1, 11, 13, 47].

Although not a focus of the study, the digestate co-product from duckweed digestion is of great importance and should be investigated in future work. Digestate can be used in a multitude of ways to provide additional value to the anaerobic digestion process, with soil amendment being a common application [8, 25, 48–50]. The high concentration of plant macronutrients (NPK) in digestate allows it to be used as a substitute for synthetic fertilizer in crop production, which helps to reduce costs and lower net greenhouse gas emissions of farming practices. Further, the carbon in digestate is quite stable and therefore enables carbon sequestration in the soil. In future work, the various technologies that are under development to enhance digestate fertility and stability should be assessed for efficacy with duckweed digestate.

Kinetic Modeling for the Thermophilic Anaerobic Digestion Process

First-order, modified Gompertz equation, transference, and logistic function kinetic models were fit to the biochemical methane data to evaluate the performance of the batch test and model the anaerobic digestion of duckweed biomass. Figure 3 displays the cumulative methane production rates (mL CH_4 g^{-1}VS) for the three duckweed varieties with the fitted models.

Table 2 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 Gompertz equation, transference, and logistic function models was set to zero because there was no observed lag time. Lag time would be a necessary parameter in studies using a non-acclimated inoculum. This experiment used inoculum directly from steady-state anaerobic digesters processing each feedstock at thermophilic conditions, so there was no lag phase. As shown in Table 2, the differences (Δ) between the predicted and observed methane productions for the three duckweed feedstocks were very small (0.03–5.43%) for all kinetic models, indicating that all four models can be used to accurately estimate the methane produced from duckweed biomass, which is in agreement with the adjusted-R^2 results [24]. 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 varieties. This is the same conclusion determined by Kafle and Chen in their evaluation of five different manure streams for BMP [38]. However, it is important to note that the first-order kinetic model is limited in that the k, rate constant, remains constant throughout the entire process, which is unlikely in a dynamic anaerobic digestion system, and the model is not suitable for complex, multi-reactant, systems. This system was simple (one reactant per batch reactor) and the use of the first-order model was appropriate. The transference model also displayed a suitable fit in this study for all three duckweed varieties 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 equation in their investigation of okra waste anaerobic digestion. The hydrolysis rate constants determined by the first-order kinetic model were 0.205, 0.285, and 0.222 day^{-1} for the OxNC, 8678, and 7741 duckweed varieties, respectively, indicating that the 8678 duckweed experienced the fastest rate of digestion. These results fit within the range observed in the literature of 0.1196–0.43 for manure-based anaerobic digestion systems [38, 51, 52]. 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 [27]. Further evaluation of the model accuracy can confirm this conclusion.

The estimated methane yield and R^2 values are not the only parameters necessary to judge the goodness of fit for

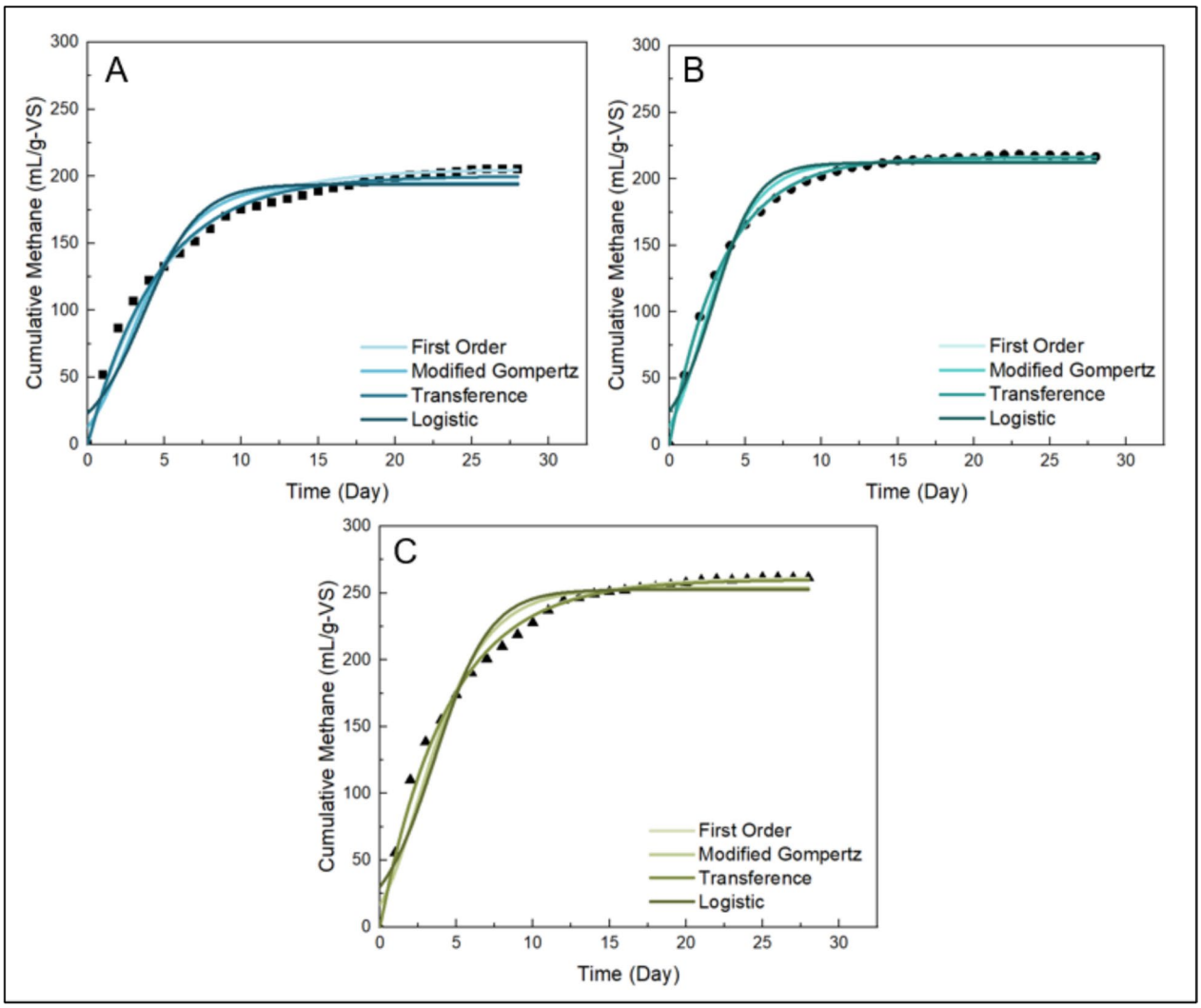


Fig. 3 Kinetic modeling results for OxNC (**A**), 8678 (**B**), and 7741 (**C**) methane production using first-order, modified Gompertz equation, transference, and logistic function models

each kinetic model. To conduct a deeper investigation of the kinetic models, this study also reviewed statistical indicators including RMSE, RSS, RSE, the AIC and the BIC (see Table 3) [24, 43]. For all of the evaluated criteria, a lower value is preferred. As shown in Table 3, the first-order, transference, and logistic function models had RMSE < 10 for all three duckweed varieties, which supports a conclusion that they are close predictors for model parameters [53]. The RMSE, RSS, AIC, and BIC values were the lowest for the first-order kinetic model for the 8678 and OxNC duckweed varieties. This was followed by, in ascending order, the transference model, the logistic function model, and the modified Gompertz equation. Note that the RMSE for the logistic function model produced from the 8678 data was lower than that of the transference model, but the RSS, AIC, and BIC values were lower for the transference model. Unlike the OxNC and 8678 varieties, the 7741 duckweed data was best predicted by the transference model, followed by the first-order kinetic model, the modified Gompertz equation, and lastly the logistic function 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 the transference and first models had the closest fits to the observed data, and supports the previous conclusions made regarding the degradability of the duckweed biomass. All kinetic models applied to this study are limited in their ability to predict or model dynamic system changes or perturbations during anaerobic digestion. The response to perturbations was not the focus of this study but should be investigated using relevant sensitive kinetic models in the future.

Previous studies that conducted the kinetic modeling of duckweed-based anaerobic digestion used secondary feedstocks (waste activated sludge and food waste materials)

Table 2 Summary of the kinetic parameters from the models for thermophilic anaerobic digestion of duckweed biomass

Model	Parameter		Unit	Duckweed Variety		
				OxNC	8678	7741
First Order	Methane Yield (M_u)	Measured	mL $g^{-1}VS_{fed}$	205.3 ± 5	216.7 ± 5	261.6 ± 7
		Predicted	mL $g^{-1}VS_{fed}$	204.7	216.6	261.1
		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 Equation	Ultimate Methane (M_u)	Measured	mL $g^{-1}VS_{fed}$	205.3 ± 5	216.7 ± 5	261.6 ± 7
		Predicted	mL $g^{-1}VS_{fed}$	194.9	213.2	254.1
		Δ	%	5.05	1.59	2.89
	Ultimate Methane Production Rate (R_m)		mL CH_4 $g^{-1}VS_{fed}$ Day^{-1}	28.80	39.27	37.76
	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 $g^{-1}VS_{fed}$	205.3 ± 5	216.7 ± 5	261.6 ± 7
		Predicted	mL $g^{-1}VS_{fed}$	200.1	216.4	260.1
		Δ	%	2.52	0.12	0.57
	Ultimate Methane Production Rate (R_m)		mL CH_4 $g^{-1}VS_{fed}$ Day^{-1}	44.33	62.59	58.79
	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 $g^{-1}VS_{fed}$	205.3 ± 5	216.7 ± 5	261.6 ± 7
		Predicted	mL $g^{-1}VS_{fed}$	194.2	212.2	252.6
		Δ	%	5.43	2.05	3.44
	Ultimate Methane Production Rate (R_m)		mL CH_4 $g^{-1}VS_{fed}$ Day^{-1}	26.74	36.95	35.21
	Lag Time (λ)		Day	0	0	0
	R^2			0.965	0.997	0.951
	Adjusted R^2			0.965	0.997	0.949

and only reviewed the modified Gompertz equation. Navarro et al. (2022) found that the modified Gompertz equation had a very close fit ($R^2 \geq 0.99$) to their experimental data from duckweed and food waste co-digestion, which reported a larger methane production (approximately 360 mL CH_4 $g^{-1}VS$) than this study. However, they 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 $g^{-1}VS$ observed in this study. This difference could possibly be attributed to the longer duration of the BMP (55 days) which was likely necessary for the completion of a mesophilic BMP test, 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 test to be completed in 28 days. Gaur and Suthar (2017) reported lower R^2 results from their use of the modified Gompertz equation (0.90–0.94) but still concluded that the fit was sufficient to validate experimental data and choose an experimental variety 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 BMP test and those estimated from the transference model used in this study. Both of the aforementioned studies that evaluated the kinetics of duckweed-based anaerobic digestion only assessed the efficacy of the modified Gompertz equation, and thus other models with more rigorous statistical analysis could prove to be better predictors of the experimental data.

This study, unlike many of the aforementioned batch digestion experiments, was conducted using available acclimated inoculum from thermophilic steady-state continuous digesters processing duckweeds and swine wastewater. The use of an acclimated inoculum is an advantage in that there was no expected or observed lag phase in the batch digestion reactors because the microbial consortia did not need to adapt or adjust to a foreign feedstock; the inoculum had been processing duckweed biomass for over

Table 3 Analysis of fit criteria for kinetic models for thermophilic anaerobic digestion of duckweed biomass

Model	Statistical Indicator	Duckweed Variety		
		OxNC	8678	7741
First order	RMSE	3.27	1.46	4.65
	RSS	683	59.3	606
	RSE	5.0	1.5	4.7
	AIC	93.8	25.2	92.6
	BIC	95.0	27.5	94.9
Modified Gompertz	RMSE	11.7	5.82	12.3
	RSS	3720	3770	4080
	RSE	11.7	11.8	12.3
	AIC	143	146	148
	BIC	144	148	150
Transference	RMSE	5.88	5.57	4.64
	RSS	933	995	580
	RSE	5.9	6.1	4.63
	AIC	103	105	89.0
	BIC	104	109	93.6
Logistic	RMSE	7.51	2.54	15.07
	RSS	5260	6440	6130
	RSE	14.0	15.4	15.1
	AIC	153	159	157
	BIC	154	163	162

150 days. Without a lag phase, the transference equation (Eq. 5) can be simplified (see Eq. 6):

$$M(t) = M_u\left(1 - \exp\left(-\frac{R_m \times t}{M_u}\right)\right) \quad (6)$$

This simplified equation is the same mathematical operation as the first-order model, which employs the first-order constant $k(\text{day}^{-1})$ in place of the R_m/M_u which represents the maximum methane production rate (mL gVS^{-1} day^{-1}) divided by the ultimate methane production (mL g-VS^{-1}). Therefore, from the transference model, it can be implied that the first-order rate constant k is equal to R_m/M_u and that in systems without a lag phase, the transference model is equal to the first-order model. A limitation of this study, and something that should be of focus in further research, is the difference in net methane yield from the thermophilic batch digestion of duckweed with and without the use of acclimated inoculum. It would also be of interest to conduct a similar study in parallel at mesophilic (35°) conditions.

The techno-economic analysis (TEA) of cultivating, harvesting, and anaerobically digesting duckweed into biogas is of great importance and should be investigated further. Calicioglu et al. conducted a TEA for the production of ethanol from duckweed and estimated the minimum selling price to be $8.23 per gallon, which is considerably higher than current market price (~$2.0 per gallon) [54, 55]. The major costs included the capital cost of the ponds (56% of total capital cost) and the operating cost of harvesting the duckweed (90% of operating cost). Notably, an anaerobic digestion system would have considerably lower harvesting costs, as compared to an ethanol process, because no water removal, pretreatment, or detoxification steps are required for anaerobic digestion of duckweed. Similarly, Osama et al. (2020) found capital costs of pond construction to be significant, which warrants further investigation into pond/reactor designs that reduce costs [56].

Conclusions

This study is the first to validate and kinetically parameterize the thermophilic anaerobic digestion of duckweeds. The inoculum used in this BMP assay was from acclimated continuous reactors, thereby eliminating the lag phase from relevant kinetic models. Kinetic modeling was conducted and determined reaction parameters including maximum methane production rates and hydrolysis constants. For the three duckweed varieties, the first-order and transference models had the best fits to the experimental data. Future work should utilize the selected kinetic models to efficiently engineer scaling-up of the thermophilic anaerobic digestion of duckweeds for biogas production via a continuous digestion system.

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Author Contribution Lillian Lower contributed to conceptualization, methodology, validation, formal analysis, investigation, data curation, writing — original draft, writing — review and editing. Yaojing Qiu contributed to methodology and computation. Ryan C Sartor contributed through resources, funding acquisition, project administration, writing — review and editing. William Joe Sagues contributed to resources, funding acquisition, project administration, writing — review and editing. Jay J. Cheng contributed through conceptualization, methodology, resources, supervision, project administration, funding acquisition, writing — review and editing.

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Data Availability The data generated during the study are available from the corresponding author on reasonable request.

Declarations

Competing Interests The authors declare no competing interests.

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