Comparison on batch anaerobic digestion of five different livestock manures and prediction of biochemical methane potential (BMP) using different statistical models

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Abstract

There is a lack of literature reporting the methane potential of several livestock manures under the same anaerobic digestion conditions (same inoculum, temperature, time, and size of the digester). And there is no literature reporting biochemical methane potential (BMP) predicting models developed and evaluated by solely using different livestock manure tests results. The goal of this study was to evaluate the BMPs of five different livestock manures (dairy manure (DM), horse manure (HM), goat manure (GM), chicken manure (CM) and swine manure (SM)) and to predict the BMPs using different statistical models. Nutrients of the digested different manures were also monitored. The BMP tests were conducted under mesophilic temperatures with a manure loading factor of 3.5 g volatile solids (VS)/L and a feed to inoculum ratio (F/I) of 0.5. Single variable and multiple variable regression models were developed using manure total carbohydrate (TC), crude protein (CP), total fat (TF), lignin (LIG) and acid detergent fiber (ADF)), and measured BMP data. Three different kinetic models (first order kinetic model, modified Gompertz model and Chen and Hashimoto model) were evaluated for BMP predictions. The BMPs of DM, HM, GM, CM and SM were measured to be 204, 155, 159, 259, and 323 mL/g VS, respectively and the VS removals were calculated to be 58.6, 52.9, 46.4, 81.4, 81.4%, respectively. The technical digestion time (T_{80-90} , time required to produce 80-90% of total biogas production) for DM, HM, GM, CM and SM was calculated to be in the ranges of 19-28, 27-37, 31-44, 13-18, 12-17 days, respectively. The effluents from the HM showed the lowest nitrogen, phosphorus and potassium concentrations. The effluents from the CM digesters showed highest nitrogen and phosphorus concentrations and digested SM showed highest potassium concentration. Based on the results of the regression analysis, the model using the variable of LIG showed the best ($R^2 = 0.851$, $p = 0.026$) for BMP prediction among the single variable models, and the model including variables of TC and TF showed the best

prediction for BMPs ($R^2 = 0.913$, $p = 0.068-0.075$) comparing with other two- variable models, while the model including variables of CP, LIG and ADF performed the best in BMP prediction ($R^2 = 0.999$, $p = 0.009-0.017$) if three-variable models were compared. Among the three kinetic models used, the first order kinetic model fitted the measured BMP data best ($R^2 = 0.996-0.998$, $rRMSE = 0.171-0.381$) and deviations between measured and the first order kinetic model predicted BMPs were less than 3.0%.

Keywords: Anaerobic digestion, Biochemical methane potential (BMP), Livestock manure, Regression model, Kinetic model

1. Introduction

The increase in production and concentration of intensive livestock operations along with the increased urbanization of rural regions have resulted in a greater awareness and concern for the proper storage, treatment, and utilization of livestock manure. Livestock manure is one of the main emission sources of the greenhouse gases. Manure-based methane (CH_4) has been estimated to contribute 4% of all anthropogenic CH_4 (USEPA, 2006). In the years to come the quantities of manure to be handled are expected to rise sharply, especially in Asia, Africa, and America. This fact, along with the fuel value of methane, calls for urgent efforts worldwide to find ways and means to capture manure-derived methane for use as an energy source (Tauseef et al., 2013).

Anaerobic digestion (AD) of livestock manure can generate methane-rich biogas and reduce air and water pollution caused by animal manure. Therefore, AD has been considered as an environmentally sustainable solution for livestock manure management. Anaerobic digestion

has been adopted and integrated into animal production systems over the last century, with thousands of full-scale anaerobic digesters currently operated in the world. There are approximately 244 anaerobic digesters (dairy -199, beef-8, dairy and beef-8, poultry-6, swine-39) systems operating on commercial livestock farms in the United States and most of them use animal manure as a sole feedstock (AgSTAR-EPA, 2014).

With the increasing interests in AD of organic materials for biogas production in the last few years, there were many efforts aiming to deepen AD knowledge and to broaden its application (Schittenhelm, 2008). To achieve the correct approach in operating AD processes, it is crucial to know the potential biogas production of a feeding organic mixture. This will optimize the process and management variables (Schievano et al., 2009). Anaerobic bio-gasification potential assay, also known as biochemical methane potential (BMP), can be used in evaluating biogas potential of both organic matrices composing an ingestate mixture and residual biogas in digestates. The BMP is a useful parameter commonly used for waste characterization. It determines the quantity of methane (ml/g VS) potentially produced by a waste in anaerobic conditions (Angelidaki et al., 2009; Hansen et al., 2004). But measuring BMP is a time-consuming process, as up to 60-90 days are required as a standard incubation time (Angelidaki et al., 2009; Hansen et al., 2004; Kafle et al., 2013) and thus, is not a practical management tool at an industrial scale for anaerobic digestion optimization. Reducing this time could lead to an increased production of methane by a better control of the fermentation (Lesteur et al., 2011). Therefore, it is attractive to use faster methods to predict how much methane gas possibly to be produced from a given substrate. This is especially the case when making theoretical studies without access to laboratory facilities, or when a fast prediction of the BMP from new substrates is required.

Mathematical models allow us to predict methane production from livestock manure without undertaking extensive and costly experiments. Models based on the composition of the waste have also been used for predicting the BMP. For instance, the Bushwel formula calculates the theoretical methane yield from the elementary chemical composition (C, H, O, N) determined by pyrolysis (Davidsson et al., 2007). The main problem of this formula is that biodegradable and non-biodegradable matter are both taken into account for the calculation, while only the biodegradable matter is metabolized into methane. Therefore, the methane yield is over-estimated. Not only the elementary composition, but also the component composition (carbohydrates, proteins, lipids, fibers, total carbon, total nitrogen) can be input in models (Lesteur et al., 2011). For solid waste, such models have already been built (Gunaseelan, 2009; Gunaseelan, 2007; Mottet et al., 2010; Schievano et al., 2009) with satisfying performances i.e. a root mean square error of prediction (RMSEP) of 73 ml CH₄ /g VS and bias of 54 ml CH₄/ g VS (Gunaseelan, 2007). Similarly, Eleazer et al. (1997) researched the connection between the cellulose, hemicellulose and lignin content of municipal residues to its anaerobic biodegradability and found that not only did the amount of lignin in the substrate influence the BMP but the chemical composition of the lignin itself played an important role as well. Determining BMPs through regression models is a relatively new methodology initiated within the last decade. Focusing only on some fresh lignocellulosic feedstock such as sorghum, napier grass and fruit, and vegetable solid wastes, relationships between biogas potential and single chemical parameters were established (Gunaseelan, 2009; Gunaseelan, 2007; Monlau et al., 2012; Triolo et al., 2011). However, regression models using chemical composition in relation to the methane recovery of different manures have not been investigated yet.

Due to the role of microbes in the anaerobic processes, kinetic models were commonly applied to simulate anaerobic biodegradation. Like the phase of bacterial growth, biogas production rate showed a rising curve, and a decreasing curve indicated by exponential and linear equations (Gioannis et al., 2009; Kumar et al., 2004). Understanding the kinetics of methane production from feedstock(s) is important for designing and evaluating anaerobic digesters. The first order kinetic model, Gompertz equation and Chen and Hashimoto model for describing the anaerobic digestion processes of different manures have been applied successfully to anaerobic treatment using different types of reactors (Kafle & Kim, 2013; Kafle & Kim, 2012; Kafle et al., 2013). To the best of our knowledge, no literature has reported the kinetics of methane production from the different livestock manures under the same test conditions using these three kinetic models.

Anaerobic biodegradation is a complex process. Therefore, the BMP determination procedure should be carefully planned, anaerobic optimal growth conditions should be secured and results should be carefully evaluated (Angelidaki & Sanders, 2004). The source of inoculum used plays a role in the biogas production rate and the biogas potential of substrates used. Similarly, the size of the digester and the loading amount used will have an effect on biogas production (Browne & Murphy, 2013). Thus, the same substrates might show different BMPs based on the tests conditions. Although, there is a number of literature reporting the methane productions from different livestock manures, there is a lack of research focusing on evaluating methane potential of several livestock manures under same anaerobic conditions (same inoculum, temperature, time, and digester size). And there is no literature presenting BMP statistical models developed and evaluated by using different livestock manures test results. Therefore, the aims of this study were (1) to compare BMPs of different livestock manures under the same conditions and to characterize the nutrients of effluents from different manures; (2) to develop single variable and multiple variable regression models based on chemical composition to predict the BMP; (3) to evaluate the different kinetic models for predicting the BMP and to determine kinetic parameters using the BMP test results.

2. Methods

2.1. Manures and inoculum used

Fresh solid dairy manure (DM) was obtained from a commercial dairy farms located in Jerome, Idaho (ID). Fresh horse manure (HM) was obtained from a local horse barn in Twin falls, ID. Goat manure (GM) of one week old was obtained from a commercial goat farm in Twin Falls, ID. Solid chicken manure (CM) of around one week old was obtained from a local chicken farm in Twin Falls, ID. Swine manure (SM) was obtained from a commercial swine farm in Kimberly, ID. The collected manures were stored at 4°C for a week before it was used. The anaerobic inoculum was obtained from a dairy manure based anaerobic digester operated on a commercial dairy in Jerome, ID. The characteristics of the DM, HM, GM, CM, SM and the inoculum used for the tests are shown in Table 1.

2.2. Test setup and design

Batch AD tests were conducted in duplicate using 0.5 L Duran glass bottles (hereafter called digesters) with a working volume of 0.2L. The batch digesters were maintained at 36.5°C in a temperature-controlled incubator (Model IB-01E/11E/21E, Lab Companion, Joe Tech Co. Ltd., South Korea). The manure loading factor was 3.5 g VS/L and a feed to inoculum ratio (F/I) was maintained 0.5 in all the batch digesters. The F/I ratio was based on the initial VS of the manure and inoculum. After adding the designed amounts of inoculum and manure in the batch digesters, the working volume of 0.2L was made up using tap water. The headspace of each digester was purged with nitrogen gas (99.9% purity) for approximately 2 minutes to ensure anaerobic conditions. Then digesters were tightly closed with silicone stopper and screw caps. Each digester was mixed manually for one minute daily just before measuring the headspace gas pressures. Assays with inoculums alone were also used as controls. Biogas and methane produced from the inoculums (controls) were subtracted from the sample assays. The results thus represent only the gas production from the manure.

2.3. Biogas and methane measurement and calculations

The daily biogas production of each digester was calculated from the headspace volume and pressure of the digester, as previously established by Kafle and Kim (2013) and Kafle et al. (2013). Methane concentration (CH_4 , %) and carbon dioxide concentration (CO_2 , %) in the biogas were analysed by using a gas chromatograph (GC) (Agilent 7980A) equipped with both thermal conductivity detector (TCD) and flame ionization detector (FID). A capillary column of dimension 60m x 0.32 mm was used in the GC. The column temperature was set at 35°C and held for 4 min for injection, then programmed at $30^\circ\text{C}/\text{min}$ to 150°C and held for 0.25 min. Helium gas was used as a carrier gas (3.0 mL/min) and the GC was calibrated by using a certified gas containing 60% methane, 30% carbon dioxide and 10% nitrogen. Biogas was sampled, using 5 mL gas tight syringes (Agilent Technologies, CA), from each digester's headspace for composition analysis. The measured biogas and methane volumes were adjusted to the volumes at standard temperature (0°C) and pressure (1 atm) (VDI-4630, 2006). The biogas production rates and methane content changed considerably as the batch digestion progressed. The methane content was measured daily during day 1 to day 5, 3 times per week during day 6 to day 15, 2 times per week during day 16 to day 28 and 1 time per week during day 29 to day 45. The methane content on intermediate days was linearly interpolated by the INTERP1 function in Matlab software R2011b (7.13.0.564). The corrected daily methane contents and the weighted average methane contents over the digestion period were calculated following the method described by Kafle and Kim (2013).

$$\text{CH}_4 \text{ Corr} = \frac{C_{\text{CH}_4} \times 100}{C_{\text{CH}_4} + C_{\text{CO}_2}} \quad (1)$$

where

CH_4_{Corr} = corrected methane content in the dry gas (% by volume),

C_{CH_4} = measured methane content in the gas (% by volume),

C_{CO_2} = measured carbon dioxide content in the gas (% by volume).

$$CH_4_{Corr, WA} (\%) = \frac{\sum_{i=1}^n BP_i \times CH_{4\ Cor, i}}{\sum_{i=1}^n BP_i} \quad (2)$$

Based on the weighted average corrected methane content and the interpolated data, the standard deviation (σ) was calculated in Excel software 2007 as follows:

$$\sigma = \sqrt{\frac{\sum (CH_{4\ Cor, i} - CH_{4\ Cor, WA})^2}{n - 1}} \quad (3)$$

where,

BP_i = biogas production on day i ,

$CH_{4\ Cor, i}$ = corrected methane content on day i ,

n = number of observations.

At the end of the experiment, mass removal in the form of biogas (biogas removed, BR) (Kafle et al., 2012) and TS and VS removals were also calculated using the method described by Kafle et al. (2013).

2.4. Analytical methods

Total solids and VS were determined in the well-mixed samples in triplicate following Standard Methods (APHA, 1998). A pH/mV meter (water quality meter pH kit-850086, SperScientific, Taiwan) was used to measure pH. Soluble chemical oxygen demand (SCOD), total chemical oxygen demand (TCOD), total ammonia nitrogen (TAN), total Kjeldahl nitrogen

(TKN), total nitrogen (TN), $\text{NO}_2^- \text{-N} + \text{NO}_3^- \text{-N}$, total potassium (K), and phosphate (PO_4^{3-}) were analyzed using a spectrophotometer (DR 5000, Hach, USA). The analyses of crude fibre (CF), crude protein (CP), total fat (ether extract) (TF), ash and nitrogen free extract (NFE) were performed following the methods detailed in (AOAC, 1990). Total carbohydrate (TC) was calculated by using CF and NFE values ($\text{TC} = \text{CF} + \text{NFE}$). The analyses of acid detergent fibre (ADF) were carried out following the methods of Goering and Van Soest (1970). The total volatile fatty acids (TVFA), alkalinity and TVFA/alkalinity ratio ((FOS/TAC – in German: Flüchtige Organische Säuren/Totales Anorganisches Carbonat) were measured by titration using a Biogas Titration Manager (Product # R41T114, Hach) (Kafle & Kim, 2011).

2.5. Statistical models to predict BMP

2.5.1. Linear regression models

In this study, nutrient compositions such as TC, CP, TF, LIG and ADF were used to develop simple and multiple regression models for predicting methane potentials. Equation (4) represents a single variable linear regression equation and Equations (5) and (6) are multiple variable linear regression equations.

$$Y = b_0 + b_1 \cdot X_1 \quad (4)$$

$$Y = b_0 + b_1 \cdot X_1 + b_2 \cdot X_2 \quad (5)$$

$$Y = b_0 + b_1 \cdot X_1 + b_2 \cdot X_2 + b_3 \cdot X_3 \quad (6)$$

where Y is the response variable, b_0 represents the Y-intercept, X_1 , X_2 , and X_3 are explanatory variables and b_1 , b_2 and b_3 are their regression coefficients.

2.5.2. Kinetic models

2.5.2.1. First order kinetic model

First order kinetic model is the simplest model, however, this model does not predict the conditions for maximum biological activity and system failures. Hydrolysis is often assumed to be the rate-limiting step in anaerobic digestion and based on this, researchers have modeled batch BMP data using first-order hydrolysis models and obtained valuable interpretations about hydrolysis kinetics. The following is a basic first order kinetic equation:

$$\frac{dC}{dt} = -KC \quad (7)$$

where

K = first order disintegration rate constant (1/d),

t = digestion time (day),

C = biodegradable substrate concentration (VS or COD).

Rearranging equation (7) and integrating for time = 0 to t days gives equation (8)

$$\ln \left[\frac{C_t}{C_0} \right] = -Kt$$

Or

$$\left[\frac{C_t}{C_0} \right] = e^{-Kt} \quad (8)$$

It is easier to derive the model by using the gas measurement instead of measuring C , which is difficult. The relationship between VS or COD and methane production can be described by equation (9):

$$\frac{C_t}{C_0} = \frac{G_o - G(t)}{G_o} \quad (9)$$

Substituting equation (8) into equation (9) produces equation (10)

$$G(t) = G_o \times (1 - e^{(-Kt)}) \quad (10)$$

where

C_t = final or outlet substrate concentration (mg/L),

C_o = initial or inlet substrate concentration (mg/L),

$G(t)$ = cumulative methane yield at digestion time t (mL/g VS),

G_o = methane potential of the substrate (mL/g VS),

K = methane production rate constant (first order disintegration rate constant) (1/day).

Equation (10) provides an accurate representation of the BMP results when (a) hydrolysis is the rate-limiting step, and (b) the maximum methane production at the infinite time (G_o) represents the total yield of hydrolysable VS at the beginning of the tests (Parameswaran & Rittmann, 2012).

2.5.2.2. Modified Gompertz model

The Gompertz model, which was set on an exponential relationship between specific growth rate and population density, was originally developed to fit human mortality data and it has also been used to predict organ growth (Causton, 1977). Gibson et al. (1987) modified the Gompertz model to a function that describes cell density during bacterial growth periods in terms

of exponential growth rates and lag phase duration. An assumption of methane production rate in a batch digester corresponding to the specific growth rate of methanogenic bacteria led to equation (11) (Lay et al., 1998). This equation has been identified as a good empirical non-linear regression model and commonly used in the simulation of methane accumulation.

$$G(t) = G_0 \cdot \exp \left\{ - \exp \left[\frac{R_{\max} \cdot e}{G_0} (\lambda - t) + 1 \right] \right\} \quad (11)$$

where

$R_{\max}$ = maximum biogas production rate (mL/d),

λ = lag phase (day),

t = time (day),

$e = \exp(1) = 2.7183$.

The lag phase represents the minimum time taken to produce biogas or taken for bacteria to acclimatize to the environment. The maximum methane production rate (R_m) describes specific growth rate of methanogenic bacteria. The higher the R_m is, the higher the biogas production rate is.

2.5.2.3. Chen and Hashimoto model

Built on both fundamental biochemical principles and the Contois model (Contois, 1959), the Chen and Hashimoto model (Chen & Hashimoto, 1978; Chen & Hashimoto, 1980) described in equation (12) has been recognized as a reliable tool in predictions regarding digestion of wastes with substantial TS content (Fongsatitkul et al., 2012).

$$\frac{C_t}{C_o} = \frac{K_{CH}}{HRT \times \mu_m + K_{CH} - 1} \quad (12)$$

where

HRT = digestion time or hydraulic retention time (days),

K_{CH} = Chen and Hashimoto kinetic constant (dimension less),

μ_m = maximum specific growth rate of microorganisms (1/day).

Substituting equation (9) into equation (12) results in equation (13)

$$\frac{G_o - G(t)}{G_o} = \frac{K_{CH}}{HRT \times \mu_m + K_{CH} - 1} \quad (13)$$

The equation (13) can be converted to equation (14).

$$G(t) = G_o \left(1 - \frac{K_{CH}}{HRT \times \mu_m + K_{CH} - 1} \right) \quad (14)$$

The Chen and Hashimoto model has obtained satisfied results for both the continuous and batch anaerobic digestion processes (Kafle & Kim, 2012; Kafle et al., 2013).

2.6. Statistical analysis, model parameters determination and evaluation

The Analysis of Variance (ANOVA) and least significant difference (LSD) analysis were performed at the significance (α) levels of 5% and 1% (Kafle & Kim, 2013).

Single and multiple variable regression analyses were performed using Excel Software, 2007. All the parameters of the three kinetic models were determined using nonlinear regressions. Nonlinear regression models were established by the ‘Solver’ function in Excel Software, 2007. The model predicted methane yields were plotted with the measured methane yields using an excel program.

To evaluate the models statistical indicators such as the coefficient of determination (R^2) and relative root mean square error (rRMSE) based on equation (15) were calculated (Bhattarai et al., 2012).

$$\text{rRMSE} = \left(\frac{1}{m} \sum_{j=1}^m \left(\frac{d_j}{Y_j} \right)^2 \right)^{\frac{1}{2}} \quad (15)$$

where d_j is the deviation between the j^{th} measured and the predicted values, m is the number of data points and Y_j is the j^{th} measured value.

3. Results and discussion

3.1. Batch anaerobic digestion test results

The biogas production rates for different livestock manures are presented in Fig.1. Fig. 2 shows cumulative biogas yields (mL/g VS) and methane contents in the biogas produced from different livestock manures. Biogas production started immediately on the first day in all the digesters. The peak values of daily biogas production rates were calculated to be 25, 19, 18, 47 and 64 mL/g VS days after 5, 7, 7, 6 and 3 days of digestion from DM, HM, GM, CM and SM, respectively. Approximately 90% of the biogas yield was obtained within 28, 37, 44, 18 and 17 days of digestion from DM, HM, GM, CM and SM, respectively. The technical digestion time (T_{80-90}) for biogas production (the time period for 80% to 90% of the maximum biogas production) was calculated to be in the range of 19-28, 27-37, 31-44, 13-18 and 12-17 days for DM, HM, GM, CM and SM, respectively (Table 2). The technical digestion time can be used as a HRT for continuous anaerobic digestion of these manures (Kafle et al., 2013). At the end of 90 days, the average biogas yields from the digesters were calculated to be 295, 222, 242 425, and 495 mL/g VS for DM, HM, GM, CM and SM, respectively. The LSD values for biogas yield were calculated to be 35 and 51 mL/g VS added at significance levels of 5% and 1%,

respectively (Table 3). No significant difference was found in the biogas potential of HM and GM but biogas potential of all other manures were significantly different from each other ($p < 0.01$).

Methane contents (%) increased till day 15 in all the digesters and thereafter remains almost constant (Fig. 2b). The weighted average methane contents of the biogas produced from the DM, HM, GM, CM and SM were 69.1%, 70.1%, 65.8%, 61.1% and 65.3%, respectively. The LSD values for the methane content were calculated to be 0.7% and 1.0% at significance levels of 5% and 1%, respectively. Statistically, significant differences ($p < 0.01$) in average methane contents between different manures were found. Based on the methane contents, the average methane yields from DM, HM, GM, CM and SM at the end of 90 days were calculated to be 204, 155, 159, 259 and 323 mL/g VS, respectively. Statistically, SM showed significantly higher ($p < 0.01$) methane potential compared to other manures. Theoretically, the complete anaerobic digestion of TC, TP and TF produces 375, 480 and 1001 mL/g VS methane, respectively (VDI-4630, 2006). Thus higher TP and TF contents in SM may be the reason for highest methane potential among all the manures. Asam et al. (2011) and Kafle et al. (2012) reported almost the same (325-330 mL/g VS) methane potential for SM as in our study but Browne et al. (2013) reported a lower BMP (70-99 mL/g VS). Triolo et al. (2011) reported BMP in the range of 197-417 mL/g VS for different swine and cattle manures. Similarly, the BMP of HM in our study fell in the range (68-273 mL/g VS) as reported by Wartell et al. (2012).

The calculated BR and VS removal at the end of the 90 days digestion period are shown in Table 2. Theoretically, the calculated biogas mass removal should be higher than the measured VS removal (Richards et al., 1991) but in our study BR was always lower than VS removal (Table 2). Kafle et al. (2012) and Liu et al. (2009) also reported lower BR than VS removal. The

measured VS removals showed good correlation ($R^2 = 0.929$, $p = 0.008$, relation is not shown) with BR. Similar to our study results, Kafle et al. (2012) also reported good correlation ($R^2 > 0.900$) between VS removal and BR. The VS removals were calculated to be 58.6, 52.9, 46.4, 81.4 and 81.4% for the DM, HM, GM, CM and SM, respectively. No significant difference in VS removal was found among the DM, HM and GM ($LSD_{0.05} = 12\%$, $LSD_{0.01} = 18\%$). Similarly, the CM and SM also showed no significant difference in VS removal. But VS removal for the CM and SM was significantly higher ($p < 0.01$) than that of the DM, HM and GM. The VS removals showed linear relationships with LIG contents and BMP (G_0). The correlations of VS removals with LIG contents and G_0 are shown in equations (16) and (17), respectively.

$$\text{VS removal (\%)} = 88.3 - 2.415\text{LIG} \quad (R^2 = 0.780, \text{Adj. } R^2 = 0.707, p = 0.047, \text{SE} = 9) \quad (16)$$

$$\text{VS removal (\%)} = 17.0 + 0.214G_0 \quad (R^2 = 0.873, \text{Adj. } R^2 = 0.831, p = 0.020, \text{SE} = 7) \quad (17)$$

3.2. Effluent characterization

The initial and final characteristics of the digester contents are shown in Table 4. Final pH decreased for the DM, HM and GM but remained constant for the CM and increased for the SM (Table 3). The calculated LSD was 0.05 at $\alpha = 0.05$ and 0.07 at $\alpha = 0.01$ (Table 3). No significant difference was observed in the final pH for the DM, HM and GM but final pH of the CM and SM was significantly higher ($p < 0.05$) than the other three manures. The TVFA concentration decreased and TA increased after anaerobic digestion for all the manures. The lowest and highest TVFA concentrations were measured for the GM and SM, respectively. No significant difference in TVFA concentration was observed for the DM, HM, GM and CM but it was significantly higher for the SM compared to the other four manures (Table 3 and Table 4). Final alkalinity concentration for the CM and SM was significantly higher than the other three

manures ($p < 0.01$), due to a higher content of CP in these two manures as opposed to other manures. A higher alkalinity concentration was the reason for the higher final pH of the CM and SM versus other manures. The degradation of CP will increase the ammonical nitrogen concentrations (Herrmann et al., 2011), resulting in an increase in the alkalinity and pH of the manure. The GM and SM effluents showed the highest TAN concentrations while the CM effluent showed the lowest TAN concentration. No significant difference in initial and final TAN concentrations were observed for the DM and CM but TAN concentrations significantly increased ($p < 0.05$) for the other three manures. The highest final TKN concentration was recorded for the SM followed by the CM. The TKN concentrations of the HM and SM were significantly decreased ($p < 0.05$) but no significant difference was found for the other three manures. The inorganic nitrogen ($\text{NO}_2\text{-N} + \text{NO}_3\text{-N}$) decreased significantly ($p < 0.01$) for all the manures after anaerobic digestion. Potassium and PO_4^{3-} concentrations significantly increased ($p < 0.05$) for all the manures after anaerobic digestion. The highest K and PO_4^{3-} concentrations were recorded for the SM and CM, respectively. No significant difference in the final K concentrations of the DM, HM, GM and CM was found but the SM effluent showed significantly higher ($p < 0.01$) K concentration than the other four manures. No significant difference was found in the final TCOD concentrations for all the manures. Similarly, all the manures (except the HM) showed no significant difference in the final SCOD concentration.

3.3. Results of regression and kinetic analysis

Linear regression models were developed to predict methane potential (G_0) (mL/g VS) using variables (manure compositions) including TC, CP, TF, LIG and ADF. The simple and multiple regression models developed in this study are listed in Table 5. Using the single variables, the most significant G_0 models ($p = 0.026$) were predicted by LIG, while other variables showed

poor regression ($R^2 = 0.347-0.668$) and lower significance levels ($p = 0.091-0.296$). The R^2 between G_0 and LIG was 0.851 and the deviations of the measured methane potentials from the predicted methane potential were calculated in the range of $11.4 \pm 3.7\%$ (Table 5). Triolo et al. (2011) reported a higher R^2 value (0.908) between G_0 and LIG for manures but a lower R^2 value (0.763) for energy crops. (Triolo et al. (2011)) explained that LIG affects BMP more significantly in energy crops than in animal manure because the cell walls of crops are protected by an intact lignocellulose matrix whereas, in animal manure, parts of this matrix are broken by animal digestion systems. The results of the simple linear regression also confirms that LIG controls VS destruction most significantly, which makes it possible to provide fine correlations between G_0 and LIG (Triolo et al., 2011). The G_0 is negatively correlated with LIG because LIG is not degradable. In contrast to our tests results, a very weak correlation ($R^2 = 0.334-0.460$) was reported by Xu et al. (2014) and Kandel et al. (2013) when using lignocellulosic biomass and reed canary grass, respectively.

Multiple regressing models using two and three variables were developed. The R^2 values were improved by the multiple regressing models and deviations between the measured and the multiple regressing model predicted G_0 were decreased compared to the linear regression models (Table 5). Similarly, Xu et al. (2014) also reported that their models including multiple feed stock characteristics had better regression results. Considering R^2 , p-value (<0.1 for each variable) and deviation, the model with the combination of two variables of TC and TF showed the best G_0 regression ($R^2 = 0.913$ and $p = 0.068-0.075$, deviations $= 7.3 \pm 5.0\%$) among the two variable models. Similarly, the model with the combination of CP, LIG, and ADF showed the best G_0 regression ($R^2 = 0.999$ and $p = 0.009-0.017$, deviations $= 0.3 \pm 0.2\%$) among models with three variables followed by the combination of TC, CP and TF ($R^2 = 0.999$, $p = 0.045-0.073$,

deviations = $1.0 \pm 1.1\%$) and the combination of TC, TF and ADF ($R^2 = 0.999$, $p = 0.042-0.081$, deviations = $1.1 \pm 1.0\%$) (Table 5).

Table 6 summarizes the estimated parameters of the studied kinetic models. Among the three models used, the first order kinetic model showed the lowest difference (0-2.5%) followed by the modified Gompertz model (2.0-4.6%). Chen and the Hashimoto model showed the highest difference between the measured and predicted methane yield (6.4-10.7%). The low deviations obtained between the theoretical and experimental values (nearly equal to or lower than 10%) suggest that all three proposed models predict the behavior of the reactors very accurately (Raposo et al., 2009). No lag phase (λ) was recorded for each manure. The use of active inoculum and the availability of soluble degradable materials in each of the livestock manures can be the reason for zero lag phase. The k , R_m , μ_m parameters of different models showed similar relation with the different manures (Table 1 and Table 6). These parameters showed an increasing trend for the decrease in LIG contents within the manure. The highest k , R_m and μ_m values were estimated for the SM followed by the CM and the lowest was for the GM. No significant difference in k and μ_m values were found for the CM and SM but the predicted R_m value for the SM was significantly higher than that for the CM. To evaluate the soundness of the kinetic model results, the predicted values of methane yields were plotted against the measured values as shown in Fig. 3, Fig. 4 and Fig. 5, respectively for the first order kinetic model, modified Gompertz model and Chen and Hashimoto model, respectively. The statistical indicators (rRMSE and R^2 values) are also shown in respective Figures. The lower rRMSE (0.171-0.381) and higher R^2 value (0.996-0.998) were calculated for the first order kinetic model followed by the modified Gompertz model (rRMSE = 0.157-0.616, $R^2 = 0.992-0.995$). Thus, based on the kinetic study results (difference between predicted and measured methane yield,

and statistically indicators) the first order kinetic model was found to be the best model to fit the measured methane yields. Zero lag phase for all the manure used in our study was one of the main reason why that first order kinetic model showed a better fit than the modified Gompertz model. In the BMP tests conducted by Kafle and Kim (2013) and Kafle et al. (2013) a lag phase was reported for their tested substrates and they reported the modified Gompertz model was a better model to predict the BMP compared to the first order kinetic model.

4. Conclusions

The BMP and VS removal for five different livestock manures were measured in the ranges of 155-323 mL/g VS and 46.4-81.4%, respectively. The highest and lowest BMP was measured for the SM and the HM, respectively. The hydraulic retention time for anaerobic digestion under mesophilic temperatures are suggested in the ranges of 19-28, 27-37, 31-44, 13-18, 12-17 days for DM, HM, GM, CM and SM, respectively. Among the five different manures used the CM digester effluents showed higher nitrogen and phosphorous concentrations and the SM digester effluents showed higher potassium concentrations than other manures. The HM digester effluents showed the lowest nitrogen, phosphorus and potassium concentrations. LIG based model was the best single variable model and the TC and TF based model was the best two variable model in terms of predicting BMP. The CP, LIG and ADF based model showed the best among the three variable models for predicting BMP. Among the three kinetic models used, the first order kinetic model was found to be the best model ($R^2 = 0.996-0.998$, deviations <3%) for predicting BMP for livestock manures.

Acknowledgements

Funding for this project was provided, in part, by the Idaho Agricultural Experiment Station and the USDA-NIFA.

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Fig.1 Daily biogas production from different manures. The values are means $\pm$ standard deviations (vertical bars, n = 3rd deviations).

Fig.2 (a) Cumulative biogas yield; (b) methane contents for biogas produced from different manures. The values are means $\pm$ standard deviations (vertical bars, n = 3rd deviations).

Fig.3 Measured methane yield plotted with methane yield predicted using first order kinetic model.

Fig.4 Measured methane yield plotted with methane yield predicted using modified Gompertz model.

Fig. 5 Measured methane yield plotted with methane yield predicted using Chen and Hashimoto model.

Table 1 Characteristics of five different manures and inoculum.

Feed	Units	Manures					
		DM	HM	GM	CM	SM	Inoculum
TS	%	16.91(0.10)	24.97(0.06)	81.63(0.27)	67.84(0.47)	31.02(0.29)	2.831(0.02)
VS	%	10.25(0.19)	18.61(0.04)	64.23(0.19)	47.5(0.26)	26.93(0.29)	1.06(0.01)
pH		8.16(0.03)	8.24-8.26	8.13(0.03)	7.95(0.04)	7.87(0.03)	7.98(0.02)
Crude protein (CP)	% TS	8.59	12.14	14.70	27.00	26.80	-
Crude Fiber (CF)	% TS	25.00	38.50	36.77	15.59	20.35	-
Total Fat(TF)	% TS	2.56	3.47	3.33	1.01	9.40	-
Total carbohydrate (TC)	% TS	46.87	59.75	61.49	43.19	51.15	-
ADF	% TS	48.31	50.98	39.70	18.21	24.42	-
Ash	% TS	41.98	24.64	20.48	28.8	12.65	-
C/N ratio		25:1	23:1	20:1	10:1	12:1	-
Lignin (LIG)	%TS	9.33	18.14	13.68	5.07	3.81	-

DM: Dairy manure; HM: Horse manure; GM: Goat manure; CM: Chicken manure; SM: Swine manure

TAN: Total ammonical nitrogen; TN: Total nitrogen

Value in parenthesis represent standard deviation (n=3)

Table 2 Measured biogas and methane potential, TS and VS removal, and biogas production duration for different animal manures.

Parameters	Units	Manure				
		DM	HM	GM	CM	SM
Biogas potential	mL/g VS	295(18)	222(5)	242(13)	425(17)	495(20)
Methane potential	mL/g VS	204(12)	155(2)	159(9)	259(9)	323(13)
Methane contents	%	69.1(0.2)	70.1(0.4)	65.8(0.0)	61.1(0.5)	65.3(0.0)
TS removal	%	50.0(6.2)	46.9(5.5)	38.7(7.3)	69.0(4.0)	74.3(1.6)
VS removal	%	58.6(6.1)	52.9(6.1)	46.4(7.1)	81.4(4.4)	81.4(2.4)
Biogas removed (BR)	g/g VS added	0.304(0.019)	0.226(0.006)	0.259(0.014)	0.478(0.022)	0.532(0.021)
T ₈₀	Days	19(4)	27(1)	31(1)	13(1)	12(1)
T ₉₀	Days	28(4)	37(2)	44(2)	18(1)	17(1)

DM: Dairy manure; HM: Horse manure; GM: Goat manure; CM: Chicken manure; SM: Swine manure

T₈₀: Duration for 80% of biogas production

T₉₀: Duration for 90% of biogas production

Value in parenthesis represent standard deviation (n=3)

Table 3 Summary of statistical analysis results.

Parameters	Units	LSD		p-value
		$\alpha=0.05$	$\alpha=0.01$	
Digester performance				
Biogas yield	mL/g VS	35.00	51.00	4.02E-05
Methane yield	mL/g VS	22.00	31.00	4.65E-05
Methane contents	%	0.70	1.00	5.02E-06
VS removal	%	12	18	0.003615
TS removal	%	12.00	17.00	0.004376
BR	g/g VS removed	0.040	0.057	3.67E-05
pH		0.05	0.07	2.61E-06
TAN	mg/L	52.00	71.00	0.004822
TKN	mg/L	92.00	125.00	0.002529
NO ₂ ⁻ -N + NO ₃ ⁻ -N	mg/L	16	-	0.020729
K	mg/L	45.00	61.00	5.92E-06
PO ₄ ⁻³	mg/L	61.000	83.000	3.52E-05
TVFA	mg/L	90	124	1.43E-08
Alkalinity	mg/L	105	143	5.98E-09
TVFA/alkalinity		0.023	0.031	4.18E-09
SCOD	mg/L	207	284	9.22E-06
TCOD	mg/L	790	1080	5.83E-09

LSD: Least significant difference

TAN: Total ammonical nitrogen; TN: Total nitrogen

Table 4 Characteristics of influent and effluent for tests using different manures.

Chemical parameters		Manures				
		DM	HM	GM	CM	SM
pH	Influent	8.16(0.03)	8.10(0.03)	8.13(0.03)	7.95(0.04)	7.87(0.03)
	Effluent	7.90(0.01)	7.91(0.04)	7.91(0.02)	7.96(0.01)	7.95(0.02)
TVFA(mg/L)	Influent	707(68)	546(4)	567(7)	732(5)	828(4)
	Effluent	108(23)	75(23)	42(23)	166(59)	281(94)
TA(mg/L)	Influent	3580(42)	3434(47)	3460(42)	3805(21)	3500(14)
	Effluent	4065(64)	4110(14)	4180(113)	4340(0)	4460(42)
TVFA/alkalinity ratio	Influent	0.198(0.021)	0.159(0.001)	0.164(0.000)	0.192(0.000)	0.237(0.000)
	Effluent	0.026(0.006)	0.018(0.006)	0.010(0.005)	0.038(0.014)	0.063(0.022)
TAN(mg/L)	Influent	520(28)	470(28)	500(14)	560(14)	510(14)
	Effluent	560(14)	570(0)	620(42)	510(42)	620(42)
TN(mg/L)	Influent	1037(7)	1026(9)	1002(9)	1185(9)	1116(9)
	Effluent	975(85)	891(17)	960(21)	1085(108)	995(23)
TKN(mg/L)	Influent	1005(8)	984(8)	957(8)	1152(8)	1092(8)
	Effluent	956(83)	870(17)	939(17)	1070(104)	978(25)
NO ₂ ⁻ -N + NO ₃ ⁻ -N (mg/L)	Influent	32(15)	42(17)	45(0)	33(0)	24(0)
	Effluent	19(2)	21(0)	21(4)	15(4)	16(2)
K(mg/L)	Influent	620(11)	600(28)	680(21)	680(28)	600(14)
	Effluent	760(28)	720(0)	780(28)	730(14)	845(21)
PO ₄ ³⁻ (mg/L)	Influent	441(2)	426(2)	509(2)	564(2)	463(18)
	Effluent	581(30)	564(65)	576(34)	730(8)	602(42)
TCOD(mg/L)	Influent	13750(71)	14950(71)	14200(71)	15500(141)	14600(141)
	Effluent	9000(1131)	9350(212)	10250(71)	8900(141)	8600(141)
SCOD(mg/L)	Influent	2400(71)	2400(71)	2100(141)	2200(57)	3250(71)
	Effluent	2150(71)	2450(71)	2150(212)	2100(0)	2050(71)

Values in parenthesis represent standard deviation (n=3)

DM: Dairy manure; HM: Horse manure; GM: Goat manure; CM: Chicken manure; SM: Swine manure

TAN: Total ammonical nitrogen; TN: Total nitrogen

Table 5 Regression of methane potential using explanatory variables.

Explanatory variables	Equation	R ²	Adj. R ²	SE	p-value	% difference between measured and predicted value	
						Range	Mean(SD)
Simple linear regression							
TC	G ₀ = 516.5 - 5.65TC	0.398	0.197	64	0.254	5.1-29.5	16.0(10.6)
CP	G ₀ = 98.5 + 6.82CP	0.668	0.558	47	0.091	9.0-24.9	17.3(6.7)
TF	G ₀ = 168.2 + 13.14TF	0.347	0.130	67	0.296	1.1-37.7	22.3(16.0)
LIG	G₀= 330.3 - 11.01LIG	0.851	0.802	32	0.026	5.8-15.9	11.4(3.7)
ADF	G ₀ = 362.2 - 3.91ADF	0.633	0.510	50	0.108	4.9-30.1	15.9(9.2)
Multiple linear regression							
TC, CP	G ₀ = 283.6 - 3.11 TC + 5.6 CP	0.768	0.535	49	0.216, 0.452	6.7-15.8	11.2(4.0)
TC, TF	G₀= 515.5 - 6.85 TC + 16.29 TF	0.913	0.826	30	0.068, 0.075	1.5-15.7	7.5(5.3)
CP,TF	G ₀ = 86.8 + 5.80CP + 7.54TF	0.768	0.539	49	0.197, 0.452	3.1-23.9	14.4(10.5)
TC, LIG	G ₀ = 233.9 + 2.30TC - 13.42LIG	0.876	0.752	36	0.109, 0.591	2.2-20.4	10.7(7.9)
CP, LIG	G ₀ = 260.9 + 2.43CP-8.41LIG	0.889	0.778	34	0.185, 0.496	0.3-14.1	8.8(5.2)
TF, LIG	G ₀ = 288.9 + 7.29 TF - 9.75LIG	0.947	0.894	23	0.041, 0.197	0.9-13.0	7.3(5.0)
L, ADF	G ₀ = 338.3 - 10.01L - 0.49ADF	0.854	0.709	39	0.223,0.835	7.4-15.3	11.2(3.5)
TC, ADF	G ₀ =465.3 + 2.48TC - 3.16ADF	0.686	0.372	57	0.618, 0.308	0.3-19.2	11.4(8.6)
CP, ADF	G ₀ = 130.2 + 6.03 CP - 0.48ADF	0.669	0.338	58	0.685, 0.955	9.6-25.4	17.1(6.5)
TF, ADF	G ₀ = 306.8 + 9.96TF - 3.47ADF	0.824	0.648	42	0.278, 0.145	2.2-27.1	11.4(11.2)
TC, CP, TF	G ₀ = 387.0 - 5.17TC + 3.09CP + 12.53TF	0.999	0.995	5	0.045, 0.073, 0.045	0.1-2.3	1.0(1.1)
TC, CP, LIG	G ₀ = 216.9 - 1.37TC + 1.95CP -10.37LIG	0.896	0.586	46	0.833, 0.735, 0.465	1.3-18.3	9.3(6.6)
CP, TF, LIG	G ₀ = 242.6 - 1.75CP + 6.66TF - 8.0LIG	0.966	0.864	26	0.539, 0.373, 0.251	0.4-13.8	5.2(5.8)
TC, CP, ADF	G ₀ = 65.5 - 4.59TC + 12.7CP + 4.67ADF	0.808	0.234	62	0.550,0.571, 0.725	2.7-17.9	11.1(5.6)
TC, TF, ADF	G ₀ = 485.6 - 4.80TC + 13.65TF - 1.85ADF	0.999	0.994	5	0.057,0.042,0.081	0.4-2.4	1.1(1.0)
CP, TF, ADF	G ₀ = 478.8 - 4.79CP + 12.18TF - 6.09ADF	0.838	0.351	57	0.821,0.493,0.631	1.9-30.1	10.4(12.2)
TC, LIG, ADF	G ₀ = 237.2 + 2.25 TC - 13.19LIG - 0.09ADF	0.876	0.505	50	0.747, 0.432, 0.981	2.5-20.5	10.7(7.9)
CP, LIG, ADF	G₀= -163.8 + 12.85CP-13.06LIG + 7.85ADF	0.999	0.999	1	0.015, 0.009, 0.017	0.1-0.5	0.3(0.2)
TF, LIG, ADF	G ₀ = 300.9 + 7.56TF - 8.04LIG - 0.83ADF	0.956	0.824	30	0.370,0.333,0.731	0.5-15.1	5.9(6.6)

TC: Total carbohydrate; CP: Crude protein, TF: Total fat, LIG: Lignin, ADF: Acid detergent fiber

Table 6 Summary of results of kinetic study using three different models.

Parameter		Units	DM	HM	GM	CM	SM
First order kinetic model							
First order disintegration rate constant (k)		1/d	0.084	0.0676	0.0493	0.1139	0.1196
	Measured	mL/g VS	201.9	148.2	144	257.9	321.9
Methane yield (45 days)	Predicted	mL/g VS	199.3	145.3	141.8	257.9	321.2
	Difference	%	1.3	2	2.5	0	0.2
Modified Gompertz model							
Lag phase (λ)		d	0	0	0	0	0
R_m		mL/g VS.d	11.9	6.5	5.5	19.2	25.2
Methane yield	Measured	mL/g VS	201.9	148.2	144	257.9	321.9
	Predicted	mL/g VS	195.4	141.4	138.4	252.8	312.7
	Difference	%	3.2	4.6	3.9	2	2.9
Chen and Hashimoto model							
Chen and Hashimoto kinetic constant (K_{ch})			3.1	4.2	5.1	2.5	2.7
Specific growth rate (μ_m)		1/d	0.637	0.564	0.511	0.752	0.846
Methane yield	Measured	mL/g VS	201.9	148.2	144	257.9	321.9
	Predicted	mL/g VS	183.3	132.4	129.4	241.3	301.4
	Difference	%	9.2	10.7	10.2	6.4	6.4

DM: Dairy manure; HM: Horse manure; GM: Goat manure; CM: Chicken manure; SM: Swine manure