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Predicting Bioenergy Potential from Vinasse Digestion: The VUMP Model (Vinasse Utilization for Methane Production)

Lucina Marcia Kuusisto[1], Melanie L. Sattler[2]*, Victoria C.P. Chen[3]

[1]*Department of Biological and Environmental Sciences, Texas A&M University - Commerce, 2200 Commerce St., Commerce, TX 75429 USA*
[2]*Department of Civil Engineering, University of Texas at Arlington, Box 19308, Arlington, TX 76019 USA*
[3]*Department of Industrial and Manufacturing Systems Engineering, University of Texas at Arlington, Box 19308, Arlington, TX 76019 USA*

*Corresponding author's Email: sattler@uta.edu

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ABSTRACT

Global ethanol production generates 900 to 2000 billion liters per year of a high-strength liquid waste called vinasse. Vinasse represents a substantial renewable energy resource, through anaerobic digestion to produce biogas. Although a variety of previous studies have measured biogas generation from anaerobic treatment of vinasse, no previous study has developed a general model to predict biogas production from anaerobic digestion of vinasse of any composition at a range of temperatures (mesophilic). The aim of this research was thus to build a first-order model VUMP (Vinasse Utilization for Methane Production) to predict methane generation from anaerobic treatment of vinasse from ethanol production, based on readily available inputs of initial vinasse composition and treatment temperature. Lab-scale anaerobic digesters were filled with 4 synthetic vinasse mixtures with differing initial values of chemical oxygen demand, nitrogen, potassium, phosphorous, and sulfur, operated at 3 temperatures each, for a total of 12 reactors. Based on data collected, a multiple linear regression equation (R^2 = 0.80) was developed to predict first-order methane generation rate constant *k*. The selected best-fit model for *k* varied positively as functions of temperature, initial chemical oxygen demand, and the product of nitrogen and phosphorous. Preliminary validation indicated that the model predicted methane generation from commercial vinasse within 20%.

Keywords: Anaerobic Digestion, Biogas, Ethanol, Methane

INTRODUCTION

Ethanol presents many advantages as a biofuel. However, the production of ethanol from biomass generates a high-strength liquid waste called vinasse. Per L of ethanol, 9-20 L of vinasse can be generated, depending on the feedstock (corn, sugar crops, starch crops, dairy products, or cellulosic materials) (Wilkie et al., 2000; Parnaudeau et al., 2008; España-Gamboa et al., 2011). Globally, almost 100 billion L of ethanol were produced in 2015. This generated 900 to 2000 billion L of vinasse (US Department of Energy, 2016).

Traditionally, Brazil, Australia, and other countries have disposed of vinasse by applying it as a fertilizer on agricultural land (Korndorfer and Anderson, 1997; Moraes et al., 2015). This can produce short-term benefits, because the vinasse contains nutrients like potassium, magnesium, and calcium which are needed for crops like sugarcane (España-Gamboa et al., 2011). However, over the long term, such disposal can cause severe deterioration of soil, surface water and ground water, due to vinasse's high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) (20-60 g/L and 50-150 g/L, respectively); low pH (3.5-5); and high concentrations of solids (30-70 g/L), nitrogen (300-800 mg/L), phosphorous (100-500 mg/L), and potassium (2-3 g/L) (Wilkie et al., 2000; Baez-Smith, 2006; España-Gamboa et al., 2011; Moraes et al., 2015).

Satyawali and Balakrishnan (2008) reviewed the existing status and advances in treatment methods for vinasse, and found that anaerobic treatment was the most attractive primary treatment due to 80% BOD removal. Studies have shown that anaerobic treatment reduces vinasse COD by 67–98% (Vijayaraghavan and Ramanujam, 2000). Anaerobic treatment not only reduces organic pollutants but also produces stabilized residuals that can be used as fertilizer without creating water pollution problems. Finally, anaerobic treatment produces renewable energy in the form of biogas (Mota et al., 2015). The general process of anaerobic degradation for any organic substrate can be represented as (Deublein and Steinhauser, 2008):

$$C_cH_hO_oN_nS_s + yH_2O \rightarrow xCH_4 + (c\text{-}x)CO_2 + nNH_3 + sH_2S \quad (1)$$

where

$x = 1/8 * (4c+h -20 -3n-2s)$ and $y = ¼ * (4c-h-20+3n+3s)$.

A variety of previous studies have measured methane generation from anaerobic treatment of vinasse (dos Reis et al., 2015; de Barros et al., 2017; Fu et al., 2017). However, only limited number of studies have attempted to predict or model methane generation. A few of these previous studies estimated kinetic parameters for methane production from a single vinasse digested at a

single temperature (Zieminski and Kowalska-Wentel, 2015; Albanez et al., 2016). Vinasse composition, however, varies based on the feedstock utilized to produce the ethanol, as well as the particular production process (Wilkie et al., 2000). Several previous studies modeled how methane production varied as a function of one parameter: vinasse COD/N ratio (Syaichurrozi et al., 2013), COD/sulfate ratio for sugarcane vinasse (Kiyuna et al., 2017), or ratio of sugarcane press mud to vinasse (González et al., 2017). Baez-Smith (2006) developed a method to estimate methane generation from vinasse by assuming 90% BOD removal.

No previous study, however, has developed a more general model to predict methane generation from anaerobic digestion of vinasse of any composition at a range of temperatures. It was hypothesized that the initial vinasse COD concentration would affect the methane production rate, since non-zero-order reaction rates are functions of reactant concentrations. Glucose, which is converted by microorganisms to methane, provides the COD. Other initial constituents could potentially affect microbial health and growth and thus impact reaction rates. It was also hypothesized that digestion temperature would impact reaction rates, since microbial-facilitated reactions typically increase with temperature, up to an optimal range**.**

The overall goal of this research was to test these hypotheses, and to build a model VUMP (Vinasse Utilization for Methane Production) to predict methane generation for anaerobic treatment of vinasse from ethanol production, based on readily available inputs of initial vinasse composition and treatment temperature. Specific research objectives were:

1. To develop and operate laboratory scale anaerobic reactors to study the effect of vinasse composition and temperature on methane generation over time.

2. Using the laboratory data, to develop multiple-linear regression equations for predicting first-order rate constants for methane generation in terms of initial vinasse composition and temperature.

Anaerobic systems can be designed for temperatures appropriate for mesophilic bacteria (30-40°C) or thermophilic bacteria (50-60°C). Higher temperatures increase microbial activity, with activity roughly doubling for every 10°C increase within the optimal range (Khanal, 2008). Thermophilic systems thus produce methane 25-50% faster, depending on the substrate (Henze and Harremoes, 1983). The thermophilic range also demonstrates improved pathogen destruction. However, start-up for thermophilic systems is slower, and systems are more susceptible to changes in loading variations, substrate, or toxicity (Khanal, 2008). Hence, for this research, mesophilic temperatures were chosen, since these systems operate with more stability.

To facilitate their use, the models should be based on a limited number of input parameters that are fairly easy to obtain. A more complex model may provide more accurate estimates, but at the cost of additional data inputs. First-order models are used with great success to describe many processes of interest in environmental engineering; they often represent a reasonable balance between simplicity and accuracy (Cooper, 2015). Even though they do not account for all of the multiple steps that occur in anaerobic degradation processes, first-order models are widely used. For example, the US Environmental Protection Agency's widely used Landfill Gas Emissions Model (LandGEM) is a first-order model. Accordingly, an objective of this research was to develop a first-order model to predict methane generation from anaerobic treatment of vinasse.

MATERIAL AND METHODS

Vinasse compositions tested

In this research, synthetic compositions were used to enable us to vary the range of constituent values tested, and the ratios of constituent values, to aid in building a model applicable to a wider range of vinasse compositions. An experimental design (strength 2 orthogonal array) was developed (Bose and Bush, 1952; Chen, 2001), covering the range of vinasse constituents described in the literature (Wilkie et al., 2000), but limiting the constituents to levels not toxic to methanogens. Preliminary tests were conducted using the experimental design; however, only 4 of 18 batches produced significant methane. The other vinasse compositions likely contained constituent quantities and ratios unsuitable for microbial growth. The four vinasse compositions used for full testing and model building are shown in Table 1. For comparison, vinasse constituent values reported in the literature are shown in the second row from the bottom.

Temperatures tested

To study the effect of temperature, tests were conducted at 3 temperatures (30, 35 and 40°C), spanning the range of mesophilic methanogens. Each of the 4 compositions was operated at 3 mesophilic temperatures (30, 35, and 40°C), for a total of 12 runs.

Batch reactor set-up

Experiments were conducted in 3-L glass reactor flasks. Each reactor was connected to an air-tight gas-collection bag (22-L Cali-5-Bond™ Bag, Calibrated Instruments, Inc.), as shown in Figure 1. Before filling, all reactors were sealed with silicon sealant and leak-checked. Reactors were filled with vinasse of different compositions, according to Table 1. The synthetic vinasse mixtures were prepared using appropriate concentrations of glucose, ammonia, phosphoric acid, potassium hydroxide, and calcium sulfate as sources of chemical oxygen demand (COD), nitrogen (N), phosphorous (P),

potassium (K), and sulfur (S), respectively. The pH was adjusted to within between 7.0 and 8.4 using hydrochloric acid or sodium hydroxide as appropriate, and then sodium bicarbonate was added to buffer the pH. Anaerobic-digested sewage sludge collected from the City of Fort Worth Village Creek Wastewater Treatment Plant was added to inoculate each bioreactor with an initial supply of microbes. Enough sludge was added to comprise 10-15% of the total solution volume, according to Espinoza-Escalante et al. (2008). Revised Anaerobic Mineral Medium (RAMM) (Shelton and Tiedje, 1984, modified for use with vinasse by omitting constituents with N, P, or K) was added to each batch to ensure that microbes had sufficient minor nutrients.

Figure 1. Experimental set-up

Table 1. Vinasse compositions tested

Composition	COD, mg/L	N (as NH_3), mg/L	P (as PO_4)	K	S (as SO_4)
1 - Low COD	2,600	60	7	39	34
2 – Medium COD, Low P, K	75,000	1200	7	39	580
3 – High COD	147,000	550	90	39	580
4 – Medium COD, High P, K	75,000	1200	90	1742	34
Range of values tested	2,600 to 147,000	60 to 1200	7 to 90	39 to 1742	34 to 580
Literature values [a,b]	14,000 to 147,000	56 to 13,760 (total N)	0.68 to 1,990 (total P)	38.5 to 14,500	34-9500
Values tested compared to literature	Very low to high	Low to moderate	Low to moderate	Low to moderate	Low to moderate

[a,b] España-Gamboa et al. (2012); Wilkie et al. (2000).

Reactor operation and monitoring

The reactors were operated at the 3 different mesophilic temperatures (30, 35, 40 °C) via placement in constant-temperature rooms. Magnetic stirrers were used to provide continuous mixing. The reactors were operated until methane generation ceased (5-10 days).

During the initial stages of hydrolysis and acidogenesis, sodium hydroxide was added every 3 hours so that the pH did not fall below 5, which would have caused methanogens to die. Later, the pH was adjusted less frequently, as needed. The biogas volume was measured daily by pumping the gas out of the collection bag through a standard air-grab sampler (SKC Air-check pump, model 224-44XR), which pumped the biogas at 1.0 L/min, and was connected to a calibrator (Bios Defender 510M). During the gas pumping period, the time needed to empty the gas bag was recorded. A LANDTEC-GEM 2000 PLUS with infrared gas analyzer (±3% accuracy) was used to measure the concentration of methane in percent volume. LANDTEC measurements of methane have previously been compared to those from a gas chromatograph, and found to be within 7% of the GC readings (Karanjekar, 2012).

Methane model development

Assuming methane generation is first-order, cumulative methane volume can be estimated using the following equation:

$$V = L_o(1-e^{-kt}) \quad (1)$$

Where:

V= cumulative volume of methane per liter of vinasse (mL/L),

L_o = ultimate methane potential (mL/L),

k = first-order methane generation rate constant (day^{1}),

t = time (days).

Rearranging Eq. 1 and taking the natural log of both sides gives:

$$\ln(1-V/L_o) = -kt \quad (2)$$

If $\ln(1-V/L_o)$ is plotted vs. time, the negative value of the slope gives k. Linear regression to determine k values for each experiment was performed using MS Excel software. L_o was estimated from the horizontal asymptote of the plots of $\ln(1-V/L_o)$ vs. time. When the plot did not clearly reach an asymptote, the value of L_o was chosen which gave the largest R^2 value for a regression line fit to $\ln(1-V/L_o)$ vs. time.

Based on the 12 k values (one for each bioreactor run), a comprehensive multiple linear regression (MLR) equation was developed using SAS software. Six predictor variables (temperature and the five waste components COD, N, P, K, and S) were used to estimate k as the response variable. The following steps were followed for developing each MLR equation: reviewing raw data plots and correlation analyses; developing a preliminary MLR model and checking model assumptions; conducting remedial actions, such as transformations, until the model assumptions for regression analysis were satisfied;

exploring possible interaction terms; searching for good fit MLR models; selecting the best-fit MLR model. Checks were performed for constant variance (modified Levene test), normal distribution of residuals, outlier influence (Bonferroni test), multi-collinearity (variance inflation values). Transformation of the data was not necessary since normality and constant variance tests proved to be satisfactory.

The best model was selected using the backward elimination method, best subsets method and stepwise regression method, such that all parameters were significant at $\alpha = 0.1$. The best-fit model was selected based on the R^2 value, adjusted R^2 value, Mallows C_p, and Akaike Information Criterion (AIC) or Schwarz Bayesian Criterion (SBC). Parsimonious models with high R^2 and adjusted R^2, low Mallows C_p, and low AIC or SBC were used because they represent the overall goodness of fit and avoid unnecessary predictor variables (Kutner and Neter, 2004).

RESULTS AND DISCUSSION

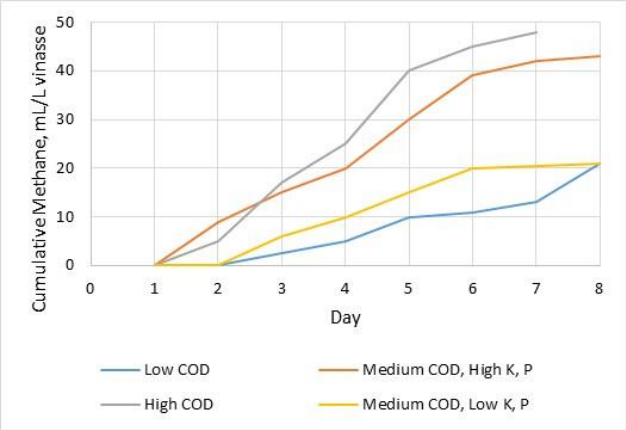
Reactor data

Figure 2 compares cumulative methane generated per liter of vinasse for the various compositions at 30°C, 35°C, 40°C. At each temperature, the composition with the lowest initial COD generates the least methane, and the composition with the highest initial COD generates the greatest methane, as expected. At each temperature, the composition with medium COD with high values of K and P generates more methane than the composition with medium COD with low values of K and P. According to the regression model discussed later, higher amounts of P are favorable for methane generation, presumably because they favor microbial growth/metabolism.

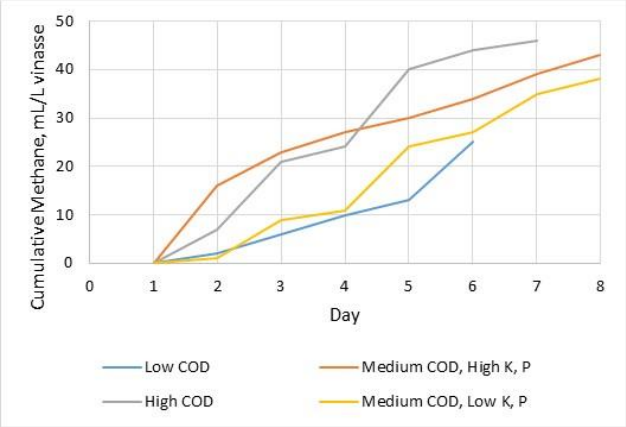
Despite the fact that the initial high COD (147 g/L) is almost double that of the medium COD compositions (75 g/L), methane generation for the high COD composition is not double that of the either medium COD composition, for any of the temperatures (with the exception of medium COD with low K and P, which had low methane generation). The medium COD compositions have higher N than the high COD composition. According to the regression model discussed later, higher amounts of N are favorable for methane generation, presumably because they favor methane growth/metabolism.

Methane generation for the composition with low COD (2.6 g/L) is higher than would be expected, considering that its COD value is only 3.5% that of the medium value, and 1.8% that of the high value, and that its N and P values are also low. This may be due to increase in the initial COD due to the sludge addition for microbial seeding.

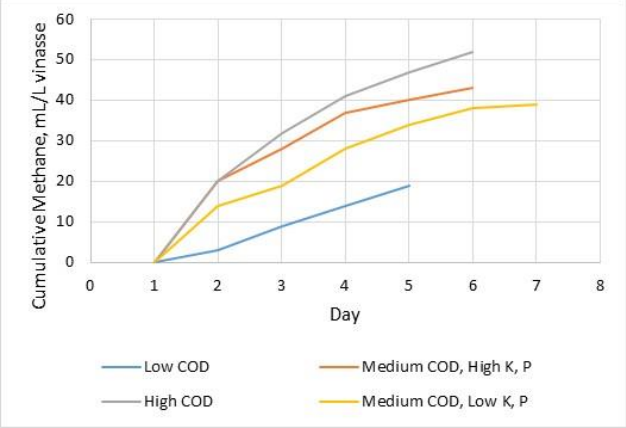
Figure 3 compares cumulative methane generated vs. time for each composition at the three temperatures. As expected, for each composition, methane generation is fastest for 40°C, intermediate for 35°C, and slowest for 30°C. For each composition (with the exception of medium COD with low K and P, which had atypically low methane generation for an unknown reason), the maximum value of cumulative methane generation is similar for the three temperatures. This is expected because the temperature impacts only the rate of methane generation. The maximum cumulative amount depends only on initial COD (glucose), which is the same for a given composition.



a) 30°C

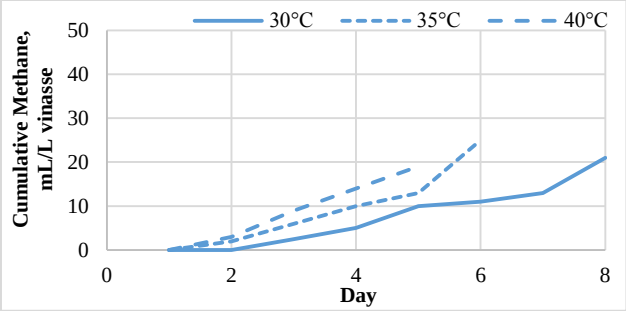


b) 35°C

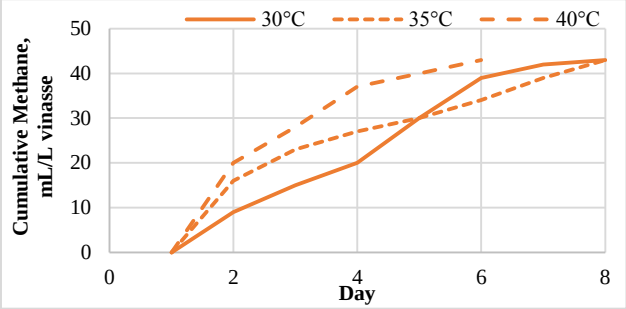


c) 40°C

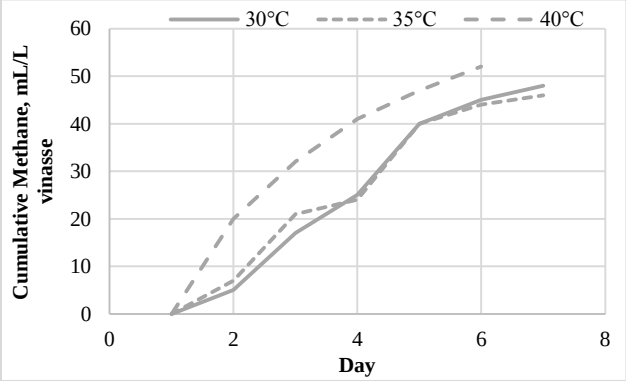
Figure 2. Cumulative methane generated from four compositions at a) 30°C, b) 35°C, c) 40°C



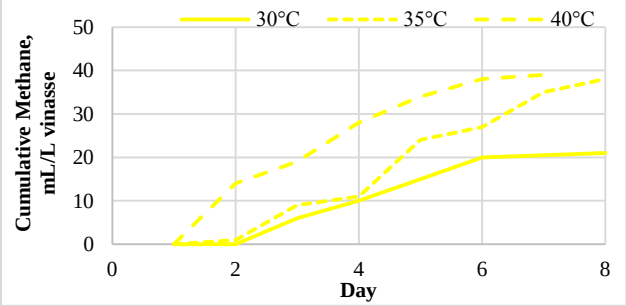
a) Composition with initial low COD



b) Composition with initial medium COD, high K, P



c) Composition with initial high COD



d) Composition with initial medium COD, low K, P

Figure 3. Cumulative methane generated at three temperatures for four vinasse compositions

Using the data for methane generation vs. time, rate constant *k* values were determined for each reactor using Eq. (2), as discussed above. Table 2 shows the *k* values for the 12 reactor runs. *k* for Composition 1 at 30°C and 35°C are shown as "N/A," because they only weakly followed a first-order trend ($R^2 < 0.75$), for reasons that are unclear. The R^2 values for the other curve fits were above 0.75 for all reactors.

Table 2. Rate constant *k* values for the anaerobic reactors

Composition	Temp. (^{0}C)	k (/day)
1	30	N/A
1	35	N/A
1	40	0.67
2	30	0.52
2	35	0.61
2	40	0.62
3	30	0.70
3	35	0.74
3	40	0.88
4	30	0.56
4	35	0.57
4	40	0.79

Model development

Using the 10 *k* values shown in Table 2, an MLR model was developed using a rigorous MLR procedure described in detail by Kuusisto (2013). The selected best model for k is shown below in Eq. 3 ($R^2 = 0.87$, adjusted $R^2 = 0.80$).

$$k = -4.96822 + 0.00243COD + 0.01757T + 0.05107(N \times P) \quad (3)$$

where:

k = methane generation first-order rate constant (day^{-1});

COD = Chemical Oxygen Demand (g/L);

T = Temperature in the mesophilic range (K);

N = Nitrogen concentration (g/L);

P = Phosphorus concentration (g/L)

All terms in the regression model were significant at $\alpha = 0.1$ level (*p*-values were less than significance level).

Real vinasse was obtained from White Energy Ethanol Distillery to perform a limited validation of the model. The vinasse solution had the following composition: COD = 3.171 g/L; N = 0.0662 g/L; P = 0.2887 g/L. The VUMP model was used to calculate a $k_{calculated}$ value of 0.452 per day. A sample of the vinasse solution was then digested at 35°C, and methane volume generated was measured over time. Using the methane volume data and Eq. 2, k_{actual} was determined to be 0.378/day. The model over-predicted the actual first-order rate constant by 20%. This was likely because the substrate used for model development was glucose, which is likely easier for microorganisms to digest than many of the compounds in real vinasse. Additional model validation is recommended.

Trends in the regression model for *k*

Eq. 3 shows that *k* increases with COD, temperature, and the interaction between N and P. *k* was not a function of K or S. The dependence on COD concentration was not surprising, given that COD is an indirect indicator of organic matter content, and organic carbon is converted to

methane in anaerobic digestion. The temperature dependence is not surprising either, given that microbial activity typically varies as a function of temperature (Khanal, 2008).

The fact that the equation was not a function of S was surprising. Kiyuna et al. (2017) found that higher vinasse sulfate concentrations decreased methane production. Barrera et al. (2014) mention the negative impact of sulfide on methanogens; although S was measured as sulfate in this study, a portion of sulfate was likely converted to sulfide by sulfate-reducing bacteria, since conditions were anaerobic.

The coefficient of the NxP term (0.05107) is larger than the coefficient of the COD term (0.00243), indicating that the methane generation rate will vary most strongly with changes in initial N or P content. Syaichurrozi et al. (2013) similarly found that methane production varied with vinasse COD/N ratio, with the optimal ratio being 600:7.

Model limitations

In this study, a model for the first-order rate constant for methane generation from vinasse was developed using lab-scale data. The model represents a first step toward the goal of being able to estimate methane generation from anaerobic biological treatment of vinasse of any composition at mesophilic temperatures, using a limited number of predictor variables (6). The model developed for *k* may not apply outside the range of vinasse constituent concentration and temperatures (30-40°C) tested in the experiments; additional work will need to be conducted to verify whether this is the case. The constituent values tested were generally low to moderate, compared to values reported in the literature (Table 1), with the exception of COD, which were very low to high. In addition, the substrate used for model development was glucose, which is likely easier for microorganisms to digest than many of the compounds in real vinasse. This led to a 20% over-prediction of the first-order rate constant, in the limited validation that was conducted.

CONCLUSIONS AND RECOMMENDATIONS

Based on data collected from lab-scale reactors, a multiple linear regression equation (R^2 = 0.80) was developed to predict first-order methane generation rate constant *k* from anaerobic digestion of vinasse. The selected best-fit model for *k* varied positively as functions of temperature, initial chemical oxygen demand, and the product of nitrogen and phosphorous concentrations. The model predicted methane generation from commercial vinasse within 20%. The next step for future work is additional validation of the model. Future work should include additional experiments and model building at thermophilic temperatures (50-60°C).

DECLARATIONS

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Authors' contributions

Lucina Marcia Kuusisto developed the experimental design, conducted the experiments, and developed the regression model. Melanie Sattler supervised Dr. Kuusisto's work and wrote this manuscript. Victoria Chen helped with the experimental design and regression model building. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent to publish

Not applicable

Ethics

Not applicable

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