2950 Niles Road, St. Joseph, MI 49085-9659, USA
269.429.0300 fax 269.429.3852 hq@asabe.org www.asabe.org

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Impact of Temperature and Mixing on Methane Production Rates of Swine Manures obtained from Deep pit Storages

Ana Paula Da Silva Batista, Undergraduate Research Assistant
Iowa State University, 3252 NSRIC, Ames, Iowa 50011, anapsbatista@gmail.com

Mark B. Van Weelden, Graduate Research Assistant
Iowa State University, 3252 NSRIC, Ames, Iowa 50011, markvw@iastate.edu

Daniel S. Andersen, Assistant Professor
Iowa State University, 3165 NSRIC, Ames, Iowa 50011, dsa@iastate.edu

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Abstract. The appearance of copious amounts of foam on the surface of deep-pit swine manure storages throughout the Midwestern United States is a serious concern for the pork industry. Manure foam has the capacity to trap gases produced by the anaerobic decomposition of the manure, leading to dangerous flammable gas concentrations upon agitation or foam disturbance. One potential cause of foaming is increased methane production from the manure. To this end, personnel at the Agricultural Waste Management Lab at Iowa State University developed a test to evaluate the methane production rate of manure. The goal of this work is to describe this assay, provide a basic summary and validation of the results it provides, and to evaluate the impact that agitation (shaken versus non-shaken) and temperature (5, 15, 25, and 35°C) had on the measured methane production rate of swine manure obtained from three swine finishing facilities in North Central Iowa. The experiment was conducted using a full factorial design with three treatments: manure source (a random variable), agitation (a fixed effect), and temperature (a fixed effect). The results indicated that the test is yielding methane production rates similar to those reported in literature. The results of the factorial experiment indicated that temperature significantly impacted the methane production rate in these tests, but that agitation did not. To better understand the impact of temperature methane production rates were plotted as a function of temperature and fitted to the Arrhenius equation. The results indicated that methane production rates approximately doubled with every 10°C increase in temperature.

Keywords. *Swine manure, methane production, foaming, temperature, anaerobic digestion, Arrhenius equation, deep-pit manure storage*

Introduction

Pork production is an important component of American agriculture, with the majority of swine production occurring in the Midwestern United States (Hatfield et al., 1998). Two of the primary environmental issues facing the swine industry are disposal of the manure and mitigation of the odors associated with both the swine production buildings and manure storages structures. As swine production systems have become larger and more specialized, these concerns have only intensified, requiring these operations to place greater emphasis on their environmental stewardship. Traditionally, swine manure has been returned to the land as a fertilizer to support crop growth (Hatfield et al., 1998). In most instances, this practice continues to this day; however, due to the prevalence of corn and soybean production, land application windows are often limited to either the spring prior to planting or after harvest in the fall. Thus, long-term storage (6-12 months) of manure is typically required on swine farms.

In Iowa, swine finishing operations typically utilize deep-pits to store the manure between land application events. These deep-pits are located within the swine production building, beneath a slatted floor on which the pigs are raised. This allows the manure to fall through to the storage pit below where it is stored until field conditions are appropriate for manure application. Storing the manure within the building limits the opportunity for rainwater dilution of the manure, minimizing manure volumes and maintaining the fertilizer value. However, the practice of storing manure within the animal housing facility has been recognized to cause several management issues, most notably maintaining adequate air quality (particularly in terms of ammonia and hydrogen sulfide concentrations) within the production facility (Zhao et al., 2005; Hatfield et al., 1998). In 2009 and 2010 swine producers began to report a new problem; foam accumulating on top of the manure pits (Moody et al., 2009; Pepple et al., 2012; Robert et al., 2011). This foam is a viscous, brown-colored layer with gas bubbles dispersed in a solids-rich liquid (Robert et al., 2011). Examples of what this foam looks like are shown in figure 1; photo a shows foam that was generated during an anaerobic incubation of swine manure in a lab setting and photo b shows foam that has accumulated on the surface of the deep-pit manure storage on a swine finishing building in Central Iowa.

(a) **(b)**

Figure 1. Manure foam accumulation on the surface of swine manure samples during (a) an anaerobic incubation of swine manure in the lab and (b) in the pumpout of a deep-pit manure storage at at swine finishing facility.

This foam represents a serious issue for pork producers as it causes the pit to fill more quickly by reducing the usable storage capacity. This can cause management problems as there may no longer be sufficient capacity to make it from one manure application window to the next, forcing the producer to apply during inopportune times or to locate alternative manure storage structures. Of even greater concern is the safety threat the foam can create. The foam bubbles are filled with biogas, which is a combination of methane, carbon dioxide, and other trace gases (hydrogen sulfide, ammonia, etc.) that result from the anaerobic fermentation of the stored manure. The foam traps and stores these potentially flammable gases (Moody et al., 2009). Activities that disrupt and break the foam, such as manure agitation or barn washing, are especially concerning as they release the trapped methane allowing it to be diluted with air to potentially explosive concentrations (Robert et al., 2011).

Numerous hypotheses have been made about what is causing swine manure to foam, including the presence of a new microbial species or changes to bacteria community structure, the increased prevalence of surface active agents, and the stabilization of the foam by hydrophobic solids (Moody et al., 2009). However, in all cases a source of the bubbles needs to be present. In manure pits these bubbles are generated from the anaerobic fermentation of the manure (methanogenesis). Thus understanding the rate at which methane is being generated by the manure could be related to factors such as how quickly the foam appears and grows,

how much foam is present on the manure storage, or even if foam is present at all. Since methane production is an important component in the generation of manure foams, a simple and quick lab-scale assay is needed to evaluate methane production rates from swine manure. To this end, personal at the Agricultural Waste Management Lab at Iowa State University developed a test to evaluate to the methane and biogas production rate of the manure. The objective of this work is to describe the methane production rate assay utilized, provide a basic summary of how the test was implemented, why this methodology was chosen, and validation of the results generated. An experiment was conducted to evaluate the impact that agitation (shaken versus non-shaken) and temperature (5, 15, 25, and 35°C) had on the measured methane production rate of swine manure obtained from three swine finishing facilities in North Central Iowa. These variables were chosen to determine if increased methane production would result from agitation of solids. We also wanted to evaluate how gas production rates would be expected to change with temperature as manure within the storage pit warms and cools during different seasons.

Materials and Methods

Description of the Methane and Biogas Production Rate Assay

Several different tests have been proposed for evaluating anaerobic digestion systems. The most well know of these is the biochemical methane potential (BMP) assay (Speece, 1996; Moody et al., 2011; Sell et al., 2011). This assay is designed to be a first-cut evaluation of the amount of organic material in a wastewater that could be converted to methane. In this test a small aliquot of the wastewater is inoculated with anaerobic biomass and incubated until gas production ceases (Moody et al., 2011). Although this test is useful for evaluating the treatability of a waste stream, it does little to indicate the rate at which methane or biogas is being produced in existing manure storages, which in the case of pit foaming would be of greater interest. Methane generation rates are often measured using barn-scale methodologies where the amount of methane emitted from the barn's ventilation system is continuously monitored or with the use of flux chambers that are periodically placed on the manure surface (Wang et al., 2010). In both cases the emission of methane from the manure can be estimated; however, these procedures are labor intensive and require substantial investment in time and equipment to conduct.

Thus, our goal was to develop a simple, lab-scale assay based on a BMP that could be utilized to quickly assess the rate at which the manure was producing methane. The procedure utilized at the Iowa State University Agricultural Waste Management Lab (AWML) is as follows. The sample of manure is agitated to achieve homogeneity. Approximately 100 g of the liquid swine manure is then poured into a clear, 250 mL graduated serum bottle (Wheaton Science Products No.:223950). The exact volume of these serum bottles should be recorded using a water displacement method; based on measurements in the AWML this volume is approximately 283.33 mL for these bottles. The exact mass of the mass of manure added to the bottle is recorded (difference in mass between the empty bottle and the mass of the manure and the bottle, graduated marks on the bottle side are used to estimate 100 mL while adding the manure). A sleeve stopper septa (Sigma-Aldrich Z564729) is then placed on top of the bottle to seal it from the atmosphere and the time the bottle is sealed recorded. The bottle is placed on a laboratory counter and incubated for three days. Biogas production was measured by inserting a needle of a glass, gas-tight syringe (Micro-Mate interchangeable hypodermic Syringe 50cc Lock Tip, Popper & Sons, Inc. New Hyde Park, New York) into the sleeve septum. Pressure in the bottle displaced the wetted barrel of the syringe. The volume of biogass extracted was read from graduated markings on the syringe body. The volume and time at which the sample was collected is then recorded and the biogas injected into an infrared gas analyzer (NDIR-CH4 Gasanalyzer University Kiel, Germany) to obtain the methane content.

The biogas and methane production rates are then calculated using equations 1 and 2. The biogas production rate (BPR) in liters of gas generated per liter of manure per day is the amount of biogas produced, equal to the volume of gas measured, corrected to standard ambient temperature and pressure (25°C, 0.986 atm), from the syringe displacement method divided by the volume of manure used in the assay and the time period of the incubation. The methane production rate is calculated similarly, but in this case the total mass of methane produced is corrected by adding the amount of biogas measured to the volume of the headspace in the bottle. This correction is required as all of the methane produced during the incubation isn't extracted, i.e., some remains in the head space of the bottle. This value is multiplied by the methane content of the biogas and then divided by the volume of manure used and the time period of the incubation.

$$BPR\left(\frac{L}{L-day}\right) = \frac{Biogas(mL) \times \rho_{manure}\left(\frac{g}{mL}\right)}{M_{manure}(g) \times time(\min)} \times \frac{1440\,\min}{day} \quad (1)$$

$$MPR\left(\frac{L\ CH_4}{L-day}\right)=\frac{\%Methane\left(Biogas(mL)+V_{headspace}(mL)\right)\times\rho_{manure}\left(\frac{g}{mL}\right)}{100M_{manure}(g)\times time(\min)}\times\frac{1440\min}{day} \quad (2)$$

This test differs from the BMP in several key ways: first, in this test the wastewater is not inoculated with anaerobic bacteria. The decision not to inoculate was based on wanting to evaluate the inherent ability of the manure to generate methane, that is, we were interested in the rate at which the endogenous bacteria could break down the organic matter in the sample to produce methane. Second, the test is a short-term incubation. The BMP incubation generally lasts about 30-45 days as it is a test of the ultimate amount of methane the sample could produce. In the case of manure foaming we aren't necessarily interested in the potential of the manure to generate methane, but rather the rate at which methane is being produced. Within the storage pit manure is continuously being added by animal excretion, this increases the amount of potential substrate for the microbes to consume. Based on the methodology utilized in this assay the addition of manure is not possible, so a short-term incubation was required to alleviate the possibility of the microbes becoming substrate limited. Finally, we generally conduct this assay at room temperature (~24°C) as opposed to the 35°C temperature used in a BMP. This temperature was selected for ease in performing the assay and because it was expected to be more representative of temperature expected of deep-pit manure storages.

Validating the Test

The first objective with this test was to validate that a three day incubation length was appropriate. This was tested by performing a series of six-day incubations where the methane production rate over the first three days were compared to the methane production rate over days four through six (methane production rates on days four through six were corrected to account for methane present in the headspace at the start of this period). Methane production rates over the first three days were plotted against methane production rates over days four through six (shown as figure 2). The generated line was then compared to the one-to-one line. The statistical analysis of the data indicated that the methane production rates over the first three days was significantly correlated to the next three days ($p < 0.0001$), that the slope of the best-fit line wasn't significantly different than one (95% confidence interval of 0.812 to 1.06) and that the intercept wasn't significantly different than zero (95% confidence interval of -0.028 to 0.012). Analysis of the residuals indicated that the distribution of residuals was approximately normal. We interpret this to mean that the methane production rate of the manure sample over a six day incubation period is approximately constant and thus the value should prove a characteristic of the manure based on its microbial population and the availability of carbon substrates in the sample.

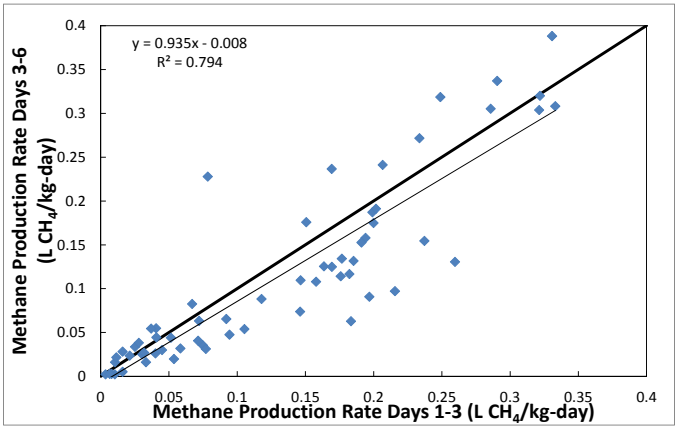


Figure 2. Comparison of methane production rates of manure samples from 62 swine finishing operations on days 3-6 and days 1-3. The thicker black line represents a one-to-one line, the thinner black line the best fit line. The best fit line was not statistically different than the one-to-one line.

Although this test provides confidence in the in the incubation period used in the assay, it doesn't test the reasonableness of the calculated methane production rates. As addressed in the introduction, barn-scale monitoring of air emissions has been used to estimate methane emission factors from swine finishing facilities. If the measured methane production rate is doing an appropriate job of estimating the inherent methane production rate of the manure, it should produce methane emission factors similar to those reported in literature utilizing farm-scale monitoring studies. However, the data is reported in very different units, so some assumptions are required to make this comparison. Specifically, the methane production rate units utilized in the assay described here are L CH_4/L manure per day, while those used in barn-scale monitoring studies are g CH_4 per head per day. To modify from L CH_4/L manure per day to g CH_4 per head per day requires numerous

assumptions. Namely assume a standard 1000 head swine finishing building with dimensions of 70 m (~225 feet) long by 13 m (~40 feet) wide, that 2.5 turns of pigs are grown per year, and that the pigs excrete manure at a rate of 4.67 L/head-day as suggested in the ASABE manure production standard. Each pig space in this barn is approximately 0.9 m^2 of floor area. If the barns are continuously stocked then this pig space would receive approximately 1700 liters of manure per year, or about 1.9 m (6.2 feet). Assuming the pit is emptied to approximately 0.305 m (1 foot) it would fill to 2.2 m (7.2 feet) by the end of the year (1 pump-out per year) and have an average depth of 1.25 m (4.1 feet). This leads us to an average manure volume of 1,125 L representing each pig space. Utilizing these approximations, the methane production rate data would suggest an emission rate of 18 ± 8 kg/finished animal, which compares favorably with the 14 kg per finished animal monitored by Pepple (2011) on a swine farm under management by the same integrator as the manures used in this study.

Experimental Design to Test Temperatue and Agitation

In this experiment the objective was to evaluate the impact agitation (shaken versus non-shaken) and temperature (5, 15, 25, and 35°C) had on the measured methane production rate of swine manure obtained from three swine finishing facilities in North Central Iowa. These variables were chosen to determine if increased methane production would result from agitation of solids. The experiment also evaluated how gas production rates would be expected to change with temperature as the manure within the storage pit warms and cools during different seasons (we have measured a low of 9°C in late winter and a high of 22°C in late summer/early fall).

The manure was obtained from three deep-pit swine finishing facilities in North Central Iowa. At the time of sampling, foam was present on the surface of two of the barns' manure pits, but not on the third. A vacuum pump was used to collect approximately 20-L of manure at each location. The manure sample was collected from the midpoint of the liquid manure depth. These farms were selected from a random population and are assumed to be representative of typical swine operations in the Midwestern United States. Total and volatile solids information on each manure sample was determined. Each sample was then split into 24 subsamples which received differing temperature and agitation treatments that were applied in a full factorial design with three replicates of each treatment (figure 3). The manure samples were incubated at 4, 15, 25, and 35°C for three days. At each temperature treatment, half of the samples were placed on a bench and not agitated, and the other half were placed on an orbital shaker set at 180 rpm. Biogas and methane production rates were assayed as described above. In the statistical analysis, temperature and mixing were considered fixed effects and manure source a random factor. The methane and biogas production rate response to temperature was then evaluated by fitting the data to the Arrhenius equation to have a model to describe temperature response.

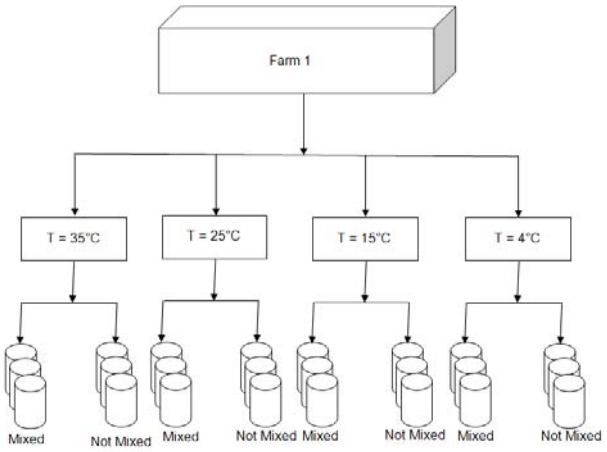


Figure 3. Schematic of the experimental design of how manure was portioned among treatments from each farm.

Statistical Analysis

Statistical analysis was performed in JMP Pro 10 as a full factorial analysis where manure source (farm) was considered a random variable, temperature a fixed factor, mixing a fixed factor, and the interaction of temperature and mixing a fixed factor. Interactions with the random variable (manure source) were pooled into the residual error term. A means separation procedure (Fisher's protected least significant difference test) was used to evaluate the significance of temperature, mixing, and the interaction of temperature and mixing on the methane and biogas production rate. The Arrhenius equation was fit to the measured data using a least squares procedure to evaluate how the biogas and methane production rates responded to temperature.

Results and Discussion

Characteristics of the manures and the biogas and methane production rates they had at 25°C are provided in table 1. These include the total solids content, the volatile solids content, the volatility of the solids (percent of solids that are volatile), the methane production rate (MPR), biogas production rate (BPR), and the methane content of the biogas (%). Of these manure samples, farm 1 came from a facility that had no foam on the manure pit when the sample was collected, whereas both farm 2 and 3 had foam present at the time of sample collection. Overall, the manure solids contents were typical of those found in deep-pit swine finishing facilities in Iowa.

Table 1. Characteristics of the manure samples used in the study of impact of temperature and agitation on methane and biogas production rates.

Manure Source	Total Solids (%)		Volatile Solids (%)		Volatility (%)		MPR (L CH_4/L-day)		BPR (L/L-day)		Methane (%)	
Farm 1	5.71	(0.02)	3.93	(0.04)	68.8	(0.5)	0.13	(0.01)	0.21	(0.01)	61.1	(5.1)
Farm 2	10.0	(0.20)	7.92	(0.16)	79.1	(0.4)	0.12	(0.01)	0.21	(0.01)	60.4	(7.5)
Farm 3	8.32	(0.06)	5.73	(0.06)	68.9	(0.3)	0.21	(0.01)	0.36	(0.02)	59.1	(3.9)

The analysis of variance table evaluating the impact of temperature, agitation, and their interaction is shown as tables 2 and 3. As can be seen from the table the interaction between temperature and mixing wasn't significant for either methane ($p = 0.0940$) or biogas ($p = 0.3809$) production nor was the impact of mixing ($p = 0.7678$ and $p = 0.3304$ for methane and biogas respectively); however, the impact of temperature was very significant ($p < 0.0001$ in both cases). In addition to the changes in methane production rates it is also valuable to note that the amount of foam that formed on the manures during the incubation varied with temperature and shaking condition. None of the shaken manures developed a foam layer, presumably due to bubble rupture from the agitation. Results for the non-agitated samples were more interesting. None of the samples incubated at 4°C developed foams, only manure from farm three developed foam at the 15°C incubation temperature, manure from both farm two and three developed foams at the 25°C incubation temperature, and all three manures developed foams when incubated at the 35°C incubation.

Table 2. Analysis of variance table for the impact of temperature and agitation on methane production rates.

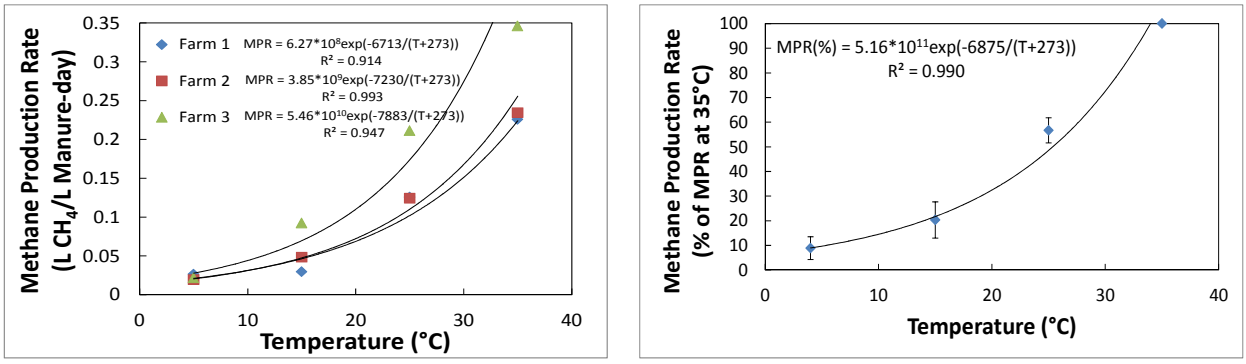
Source	DF	SS	MS	F Ratio	Prob > F
Manure Source	2	0.06494	0.03247	---	---
Temperature	3	0.65984	0.21995	315.6	<.0001
Mixing	1	0.00006	0.00006	0.088	0.7678
Temperature*Mixing	3	0.00465	0.00155	2.226	0.0940
Error	62	0.04320	0.00070		

Table 3. Analysis of variance table for the impact of temperature and agitation on biogas production rates.

Source	DF	SS	MS	F Ratio	Prob > F
Manure Source	2	0.16499	0.08250	---	---
Temperature	3	0.83993	0.27998	235.5	<.0001
Mixing	1	0.00114	.00114	0.962	0.3304
Temperature*Mixing	3	0.00371	.00124	1.041	0.3809
Error	62	0.07365	.00119		

These results did not indicate a positive feedback from agitation of the solids or an inhibition of methane or biogas release from the manures that developed foam. These results indicate that shaking should not be needed as part of the methane and biogas production assay as it did not impact the results. Moreover, not shaking the samples allowed foam to develop on top of some of the samples which could be advantageous for studying foam structure, characterizing the foam, and evaluating what is causing foam formation. The results also indicate that temperature can have a significant impact on the rate of methane and biogas production. A means separation procedure indicated that all temperature levels were significantly different ($\alpha = 0.05$) from one another. To better understand and evaluate these results the linearized Arrhenius equation was fit to the methane production rate as a function of temperature. The Arrhenius equation is a simple formula designed to describe the temperature dependence a rate constant and is shown as equation 3. In this equation k is the rate constant, T is the temperature in Celsius, and A and B are fitting parameters.

$$k\left(^{L\,CH_4}/_{L-day}\right) = A\exp\left(\frac{-B}{T+273}\right) \quad (3)$$



(a) (b)

Figure 4. Average methane production rate (a) and the average methane production rate as percent of the rate at 35°C (b) as a function of temperature with the fitted Arrhenius equation.

The data was plotted in two ways. The first, shown in figure 4 a, was to fit the measured methane production rate against temperature for each of the manures. This showed that the manure from farm three, which produced the most foam during this incubation, had the greatest temperature response. The other two manures (farm 1 and farm 2) responded similarly to temperature. The second method was to rescale the methane production rate to percent of methane production rate at 35°C (figure 4 b). This rescaling resulted in all three manures exhibiting a similar pattern of response to temperature. In general, the data was well fit by the Arrhenius equation, indicating it is an appropriate choice for modeling the response of methane production to temperature.

A common rule of thumb is that microbial activity will approximately double with every 10°C increase in temperature. Our data would suggest this approximation is reasonably valid as the methane production rate on average increased by 2.3 times from 4°C to 15°C and 2.8 times from 15°C to 25°C, but only by 1.8 times from 25°C to 35°C. Most importantly this data fit illustrates that the manure pit temperature can greatly impact the actual rate of methane production as compared to what is projected by this assay. To illustrate this point we'll refer back to the methane production rates estimated earlier, i.e., 18 ± 8 kg CH_4/head finished. Assume the manure temperature in the pit is on average approximately 15°C, then the actual rate of methane production estimated would have been 10 ± 5 kg CH_4/head finished which is more in line with actual reported methane production rates. Moreover, Pepple (2011) also reported less methane production during the colder winter/spring turn than the summer/fall turn, which is supported by the work illustrated here. Thus, using this assay to estimate methane emissions from barns would require corrections for both manure temperature and manure volume present in the barn throughout the year.

The methane content of the biogas was also calculated and is shown (figure 5). The results indicated that as temperature increased so did the methane content of the produced biogas. For most temperatures, 15-35°C, the calculated methane content was in the range that would typically be expected 40-70%. At the 4°C temperature the calculated methane content was much lower, however; this may have been due to the low amount of both biogas generated as evidenced by the low methane production rate. In evaluating this assay it should be recognized that the partial pressure of CO_2 in the headspace is controlled by the pH of the manure and total carbonate concentrations in the manure as described by Henry's Law and the chemistry to the bicarbonate system, thus CO_2 release is impacted by both thermodynamic and biological means in this assay whereas methane production would be entirely due to biological production.

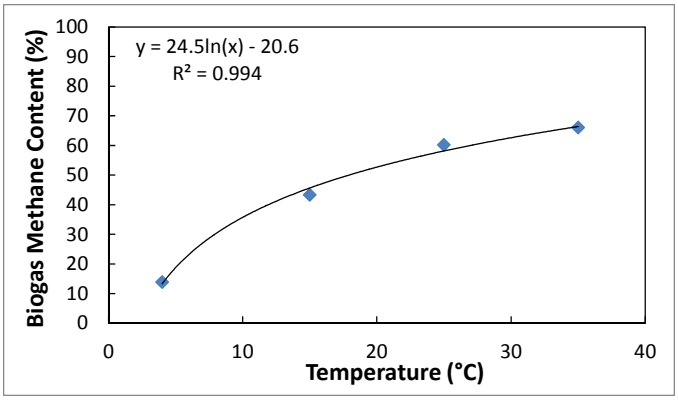


Figure 5. Methane content of the biogas as a function of temperature.

Conclusions

The appearance of foam on the surface of deep-pit manure storages is an issue of serious concern for the swine industry. The generation of methane and biogas is an important component of manure foaming. The assay used in the Iowa State Agricultural Waste Management Lab to evaluate the methane and biogas production rate of manure was described. The impact that agitation and temperature had on methane and biogas production rates on swine manure from three deep-pit swine finishing facilities in north-central Iowa was evaluated with results indicating that only the impact of temperature was significant. The Arrhenius equation was fit to the data and results indicated that the methane production rate approximately increased by a factor of 2 for every 10°C increase in temperature. The test is providing useful insight into the methane production rate of manures and should be a useful tool for studying the potential causes of manure foaming, potential mitigation strategies, and provide a method to estimate methane emissions from swine barns and manure storages.

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