

THESIS

CATTLE MANURE CHARACTERISTICS IN RELATION TO MANURE ACCUMULATION  
PERIOD AND SEASONAL IMPACTS IN THE INTERMOUNTAIN WEST

Submitted by

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## ABSTRACT

### CATTLE MANURE CHARACTERISTICS IN RELATION TO MANURE ACCUMULATION PERIOD AND SEASONAL IMPACTS IN THE INTERMOUNTAIN WEST

Anaerobic digestion (AD) of organic waste has been studied and implemented in practice more recently than ever. AD is used to produce biogas, which mainly consists of methane gas that has multiple purposes. Arguably the most important purpose methane gas has is that it is a renewable energy source. Organic waste that can serve as feedstock for AD ranges from food waste to animal waste, including manure. There are limited application of AD technology to process beef cattle manure compared to other manure sources due to factors such as method of collection processes (i.e., scraped on dry lots) and inconsistent methane gas produced due to varied conditions. This study focuses on beef cattle manure from the intermountain west, home to many cattle feedlots producing millions of tons of manure a year. Beef cattle feedlots pens typically have a base of compacted manure with no roof covering the pen. With no roof over the beef cattle feedlots and low collection frequency, the manure is exposed to seasonal change with varying weather conditions and often contains low water content and high inorganic material compared to other animal feeding operation manure. To improve the methane production of beef cattle manure in AD processes, more information is needed on the quality of beef cattle manure over varying collection frequency and seasons.

The objective of this research is to determine biochemical methane potential (BMP) for differing accumulation time periods and seasonal impact. Four sample collections were conducted at one concentrated animal feeding operation (CAFO) from different months with different seasonal effects. For each sample collection, different manure accumulation periods were selected ranging from 7 to 90-day old manure. For each accumulation period, three cattle pens were selected based on the similar number of cattle and same feed. All manure was collected from each pen and was weighed with subsequent testing for characteristics. Based on the total solids (TS) from each pen in each manure accumulation period the composite sample was created for the manure accumulation period which was tested for BMP. A second manure collection technique was used due to complications with collecting lower manure accumulation periods with the first collection technique such as an uneven surface of the pen. The second technique applied land surveying one singular pen for a 10- and 20-day manure accumulation period to attempt to only collect newly deposited manure over the accumulation period.

The BMP data is expressed as the volume of methane ( $\text{CH}_4$ ) produced per unit of volatile solids (VS) added, typically represented as  $\text{mL CH}_4/\text{g VS feedstock}$ . VS is the portion of the material that is organic and for this study in terms of volatile mass per dry mass. The sample collections from this study BMP results ranged from 200-276  $\text{mL CH}_4/\text{g VS feedstock}$ . A literature review was conducted comparing over 13 studies that tested beef manure in AD. Results from this study were above the average of the literature review which was 160  $\text{mL CH}_4/\text{g VS feedstock}$ . One of the sample collections occurred in May, which was the only data which represented a parallel trend between manure accumulation periods and BMP results, with BMP decreasing with longer accumulation periods. The May sample collection produced the highest measured ultimate BMP

and was considered the most precise manure collection. Statically different trends were not observed for samples collected over seasons, leading to inconclusive results on seasonal impacts on BMP. The land surveying technique for manure collection resulted in variable quality manure, which emphasized the struggle of replication of manure collected and the possibility of obtaining only the desired manure accumulation period without obtaining any of the manure pad. Overall, results indicated the potential for increased methane production potential for more frequently collected manure at beef feedlots. However, the study also indicated that more frequent collection of only freshly deposited manure would be difficult to implement in practice.

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## TABLE OF CONTENTS

ABSTRACT.....	iii
ACKNOWLEDGEMENTS.....	v
LIST OF TABLES.....	x
LIST OF FIGURES .....	xi
LIST OF ACRONYMNS .....	xiv
CHAPTER 1: INTRODUCTION.....	1
1.1    Research Motivation .....	1
1.2    Thesis Overview.....	3
Chapter 2: BACKGROUND AND LITERATURE REVIEW.....	5
2.1 Introduction to AD Process .....	5
2.1.1 Hydrolysis .....	6
2.1.2 Acidogenesis and Acetogenesis .....	6
2.1.3 Methanogenesis.....	6
2.1.4 Factors that Affect AD Process.....	6
2.2 Uses of Produced Biogas.....	7
2.3 Challenges for AD of Beef Cattle Manure.....	7
2.4 Assessing Anaerobic Biodegradability .....	8
2.5 Biochemical Methane Potential Test.....	8

CHAPTER 3: MATERIALS AND METHODS .....	10
3.1 Beef Feedlot Manure Management .....	10
3.2 Experiment Setup .....	10
3.2.1 Temperature and Precipitation .....	11
3.3 Manure Collection and Preparation.....	13
3.3.1 Manure Collection at from Pens .....	15
3.3.2 Land Surveying Lower Manure Accumulation Periods for Manure Samples .....	17
3.3.3 Sample Preparation .....	18
3.3.4 Selection of Representative Samples for Manure Accumulation Periods.....	20
3.4 Analytical Methods .....	20
3.4.1 Solids Characterization .....	20
3.4.1.1 Total Solids .....	21
3.4.1.2 Volatile Solids.....	22
3.5 BMP Batch Test .....	22
3.6 Inoculum & Nutrient Medium.....	25
3.6.1 BMP Cart Setup .....	27
3.7 Orbital Shaker .....	29
3.8 Determination of Sample Quantity for BMP Bottles.....	31
3.9 Gas Collection and Testing.....	32
3.10 Normalizing Cumulative Methane Production .....	34

3.11 Assessing Differences in Seasons and Manure Accumulation Periods .....	36
Chapter 4. Results and Discussion.....	37
4.1 Characterization of Solids in Manure Samples .....	37
4.1.1 Normalized Sample Collection Total Weight .....	40
4.1.2 Total Solids and Volatile Solids of the Manure Collected by the Land Surveying Method .....	41
4.2 BMP Batch Assay Results .....	45
4.2.1 Land Surveyed Samples Compared to Scraped Collection Samples .....	48
4.2.2 Manure BMP Results for Accumulation Periods Compared to Literature .....	51
4.3 Comparison of Seasonal Differences in Cattle Manure Quality for Methane Production..	52
4.3.1 Comparison of Manure Accumulation Periods for Sample Collections .....	54
4.3.3 Pairwise Comparisons of Methane Potential .....	55
4.4 Comparison of BMP Observed in this Study to Other Studies .....	57
Chapter 5. Discussion .....	61
Chapter 6. Summary .....	65
APPENDIX .....	72
Appendix A: May Sample Collection- Zones .....	72
Appendix B: 10 Year Average Temperature for Sample Collection Months .....	74
Appendix C: Bottle Leak Test Protocol.....	75
Appendix D: Bag to Bottle Leak Test Protocol .....	76

Appendix E: GC Manual..... 78

LIST OF TABLES

Table 1: Manure accumulation periods for sample collections represented by X..... 11

Table 2: Average BMP results from all sample collections by manure accumulation period..... 51

Table 3: Statical comparison of each manure accumulation period from sample collections where the duration of manure accumulation period is included in parentheses after the month of sample collection..... 56

Table 4: Represents % not significantly different and significantly different of each sample collection manure accumulation periods compared to each other seen in Table 4..... 57

Table 5: BMP results from this study compared to literature..... 59

## LIST OF FIGURES

Figure 1: The four stages of AD .....	5
Figure 2: Precipitation reported accumulated over period (e.g., precipitation for 7 day is the sum over the 7-day period) and for the sample collection months.....	12
Figure 3 reported are an average over the periods reported (e.g., temperature for 7 day is a 7-day average). Monthly averages and the 10-year average are reported on the right side.....	12
Figure 4: Zone 1 and Zone 2 in a singular pen .....	14
Figure 5: Front-end loader with no box blade .....	16
Figure 6: A singular row of manure from one pen with flag stakes placed for sample collection. .....	17
Figure 7: Selected land surveying pen initial conditions in April 2023 .....	18
Figure 8: BMP bottle setup including the red valve and tubing that will connect to a gas bag....	24
Figure 9: BMP Cart setup where BMP bottles were configured in an anaerobic system. The left container contained the inoculum and the right container contained nutrient medium. ....	28
Figure 10: Gas collection bags located outside the orbital shaker.....	30
Figure 11: BMP bottle configuration inside orbital shaker with tubing exiting on the left to gas collection bags. ....	31
Figure 12: 100 mL syringe luer lock tip with three-way-valve. ....	33
Figure 13: %TS reported for all four sample collections for each collected manure accumulation period. Error bars indicate + one standard deviation (includes three pens and three technical replicates).....	37

Figure 14: %VS reported for all four sample collections for each collected manure accumulation period. Error bars indicate + one standard deviation (includes three pens and three technical replicates)..... 39

Figure 15: Total weight of manure collected from each manure accumulation period, normalized to day 7 weight. April sample collection is not included due to it not having a 7-day manure accumulation period..... 41

Figure 16: %TS and %VS for 10-day land surveyed manure compared to similar manure accumulation periods. Error bars indicate + one standard deviation (includes three pens and three technical replicates). ..... 42

Figure 17: %TS and %VS for 20-day land surveyed manure compared to similar manure accumulation periods. Error bars indicate + one standard deviation (includes three pens and three technical replicates). ..... 43

Figure 18: Land Survey desired spot representing uneven surface. .... 44

Figure 19: BMP results with respect to VS for all four sample collections including all manure accumulation periods. Error bars indicated + one standard deviation (includes three BMP bottles and three technical replicates for methane and VS analysis)..... 45

Figure 20: BMP results with respect to TS for all four sample collections including all manure accumulation periods. Error bars indicate + one standard deviation (includes three BMP bottles and three technical replicates for methane and VS analysis)..... 48

Figure 21: Measured Ultimate BMP comparison of the 10-day land surveying data to similar manure accumulation periods from sample collections. Error bars indicate + one standard deviation (includes three BMP bottles and three technical replicates for methane and VS analysis). ..... 49

Figure 22: Measured Ultimate BMP comparison of the 10-day land surveying data to similar manure accumulation periods from sample collections. Error bars indicate + one standard deviation (includes three BMP bottles and three technical replicates for methane and VS analysis). ..... 50

Figure 23: August 2022 and May 2022 sample collection data, a symbol (A) was placed to indicate that there is no significant difference (P-value > 0.05). Error bars indicate + one standard deviation. May and August n is 4 (three technical replicates), November n is 5 (three technical replicates). April sample collection is not included on the figure due to only having one manure accumulation period..... 53

Figure 24: Statistical analysis on average sample collections manure accumulation period measure ultimate BMP. Error bars indicate + one standard deviation. The months included in each manure accumulation period varied as follows; 7- and 14-day manure accumulation periods (May, August, and November), 21-day manure accumulation period (August and November), and 48-day manure accumulation period (August, November, and April). The 28-, 56-, and 90-day manure accumulation periods are not included due to them only being in one sample collection..... 54

Figure 25: May Sample Collection Zone 1 and 2 ..... 72

Figure 26: Ultimate BMP data for the May Sample Collection ..... 73

Figure 27: 10 Year Average Temperatures for Sample Collection Months ..... 74

## LIST OF ACRONYMS

ACRONYM	DEFINITION
AD	Anaerobic Digestion
GC	HP-Agilent 8690 Gas Chromatograph
BMP	Biochemical Methane Potential
CAFO	Concentrated Animal Feeding Operation
ISR	Inoculum to Substrate Ratio
VM	Volatile Mass
DM	Dry Mass
WM	Wet Mass

## CHAPTER 1: INTRODUCTION

### 1.1 Research Motivation

Over time, humanity has continually adapted to its environment through education and technological advancements to pursue a more efficient lifestyle. This process has led to a vast number of products, materials, and resources that are accessible to many of the world's people. Consumer products require a diverse array of materials and resources including the consumption of energy. Albert Einstein once said, "Energy cannot be created or destroyed, it can only be changed from one form to another." Particularly in the United States, it is more common for people to buy new products instead of buying used products or repairing existing products in a vicious cycle known as consumerism. This cycle is driven by the fact that new products, made often of synthetic materials, are relatively cheap to produce. However, a significant amount of energy and materials are required to make all these new products, resources that are crucial for the future development of necessities for human survival, and these processes often require the use of non-renewable energy sources. Non-renewable energy is energy from a source that will run out or cannot be replenished (National Geographic, July 21,2023). As of 2022, about 80 percent of the world's energy comes from non-renewable fuel sources (NRDC, July 21, 2023). Out of that 80 percent, oil accounts for 32.8 percent, coal for 27.2 percent, and natural gas for 20.9 percent (Hook et al. 20113). Fossil fuels are the main source of greenhouse gases (GHG), as the use of fossil fuels remains a main source of energy across the globe, the price has been increasing due to depletion rates. As a result, there is a pressing need to explore and adopt alternative, more sustainable energy solutions to ensure a secure and thriving future for humanity.

One prominent category of consumer goods in the United States is food and in particular, meat. Among various animal meats, beef holds a prominent position in American diets, with the average individual consuming approximately 55 pounds of beef per year (Beef Consumption in the U.S, July 21, 2023). On a national scale, the United States consumes a staggering 27.3 billion pounds of beef annually (Statista, July 21, 2023). Regarding beef cattle farms, there is one specific byproduct that is of concern, but could also be the future of energy- methane. Methane is a significant contributor to greenhouse gas emissions and is produced mainly when cattle digest feed (UNL Water, July 21, 2023). However, harnessing methane gas presents an opportunity to mitigate greenhouse gas emission while addressing the demand for energy through renewable energy (Qi et al. 2022).

Beef feedlot manure poses several challenges for a substrate for AD. Manure often contains high levels of ash and low levels of volatile mass typically known as organic matter, that hinder the AD process (Watson et al. 2015). Comparatively low organic content results from manure degradation during large storage periods in pens. Another reason that manure collected could have low organic content and higher inorganic content, is due to the collection process gathering sand and rocks from the manure pen base (manure pad). Due to the manure having fewer volatile solids than other typical substrates for AD, more manure is required to obtain the same percentage of VS as other substrates. AD also requires a significant amount of water to optimize the solids concentration. Feedlots typically do not have covers over the pens, allowing seasonal conditions to affect the water content in the manure (Tucker et al. 2015). Other case studies referenced in this paper that used beef cattle manure as the substrate for BMP testing focused on optimal conditions for the AD process and whether mixing beef cattle manure with another substrate would yield a higher Biochemical Methane Potential (BMP). The average BMP results

from the other case studies were lower than the average BMP results for the sample collections that occurred in this study. However, there is a noticeable gap in knowledge regarding the impact of manure age (manure accumulation period) and seasonal conditions on the final BMP measurement. This research aims to address these challenges by investigating manure accumulation periods and seasonal impact through AD processes.

## 1.2 Thesis Overview

The focus of this study was to have a better understanding regarding manure characteristics on manure accumulation periods and seasonal impact. The main objectives of this research were:

- Assess seasonal impact in the intermountain west on BMP results.
- Assess impact of manure accumulation periods on BMP results.

All manure used in this study was collected from a CAFO in the intermountain west. Manure was collected (sample collections) during four different months of the year having different seasonal impacts. For each sample collection selected manure accumulations periods were collected. Each manure accumulation period was a composite sample from three different pens with the same manure age, similar number of cattle, and same feed. After the manure was collected, an intensive sample preparation process was conducted. Manure was collected from each desired pen and tested for characteristics before the combining process for the manure accumulation period. The main characteristics that were tested included percent total solids (%TS) and percent volatile solids (%VS). The purpose of testing for %TS and %VS is to understand the manure composition, assess digestibility, and operational behavior. Through testing for %TS and %VS a comprehensive understanding of the manure can be determined. Based on the %TS from the three pens the manure accumulation period was formed. Each

manure accumulation period was tested for %TS, %VS, and the ultimate BMP. The test to determine the ultimate BMP is known as a batch assay using anaerobic digestion.

## Chapter 2: BACKGROUND AND LITERATURE REVIEW

### 2.1 Introduction to AD Process

Anaerobic Digestion (AD) technology has gained significant global traction due to the economic and environmental benefits including its capabilities as a source for renewable energy. AD process involves the composition of solid waste such as sludge, bio waste, cattle manure, and other biomasses create bioenergy in the form of methane gas. AD is a complex multi-step process that involves numerous types of microorganisms which can be affected by the following factors: temperature, pH, carbon to nitrogen ratio, organic loading rate (OLR), and trace elements. AD is composed of four stages (Figure 1), with each step dependent on a biological community. Throughout this journey, complex organic matter goes through the three steps to produce biogas (Angelidaki et al. 2004).

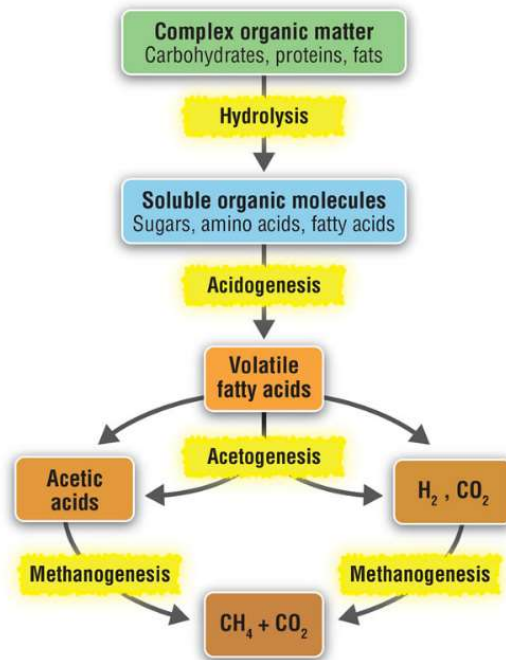


Figure 1: The four stages of AD

### 2.1.1 Hydrolysis

Hydrolysis is an important step of the anaerobic biodegradation process of complex organic matter. Hydrolysis is the first step in AD and often is the rate-limiting step, using substrates with high solid contents. An important factor for hydrolysis is the physical state and structure of the substrate and the accessibility for hydrolytic enzymes. During hydrolysis, organic substrates are broken down into smaller products that can subsequently be taken up and degraded by bacteria. An example of this conversion includes complex compounds such as carbohydrates, proteins, and lipids, which then become glycerol and long chain fatty acids (Angelidaki et al. 2004).

### 2.1.2 Acidogenesis and Acetogenesis

Acidogenesis and acetogenesis both occur in step two, which is part of fermentation. The smaller products produced in hydrolysis then interact with the acidogenesis bacteria, which then converts the product into a volatile fatty acid (VFA). Next, the acetogenesis bacteria complete the conversion to hydrogen, carbon dioxide, and acetic acid.

### 2.1.3 Methanogenesis

The last phase of AD is the methanogenesis stage, in which methanogens convert the products produced previously into insoluble methane and carbon dioxide. During this stage, the pH of the conversion process will rise to neutral values ranging from about 6.8-8 (Zamri et al. 2007.).

### 2.1.4 Factors that Affect AD Process

When a substrate is biodegrading there are several chemical and physical factors in the environment that can affect the process. Factors include the availability of the compounds, availability of electron donors and acceptors, pH, salinity, oxygen concentration, temperature, and concentration of chemicals. However, different factors are dependent on the substrate characteristics (Angelidaki et al. 2004).

## 2.2 Uses of Produced Biogas

The main biogases produced from AD of cattle manure are methane ( $CH_4$ ) and carbon dioxide ( $CO_2$ ). The methane produced has several uses, the most relevant one for today's society being renewable energy. Methane can be used directly as a fuel for electricity generation, heating, and combustion in engines to produce mechanical power. Biogas can be a substitute for natural gas within industrial buildings and homes. The methane produced can also be injected into the natural gas grid. California Senta Bill 1440 aims to use renewable natural gas for a portion of their gas utilities. Representing the future of renewable natural gas in the United States.

The utilization of biogas from cattle manure using AD can help reduce the large amount of greenhouse gas emissions. Biogas production helps mitigate odors and other potential environmental impacts associated with manure storage management (Nishio and Nakashimada, 2007).

## 2.3 Challenges for AD of Beef Cattle Manure

Beef cattle manure has characteristics that offer challenges for processing using conventional AD technology. As stated previously, manure is generated in outdoor pens with no covers, allowing the manure to be impacted by seasonal conditions. The manure can range from being dry to wet or even frozen. An important challenge beef cattle manure faces is the potential for low organic solids and fiber contents which results in low biogas yield and degradation efficiency (Eliasson et al. 2021). A key factor in the methane generation potential of manure is the temperatures manure is exposed to during time in the pen. Lower temperatures can lead to slow digestion in the pen but higher BMP results when tested (Yao et al. 2020). Beef cattle manure tends to have inorganic solids in it when collecting such as sand and rocks. The sand and rocks create challenges for conventional AD systems. Some of the challenges that could occur due to the

inorganic material include reducing the residence time of the manure and can potentially decrease biogas production rate (Sharvelle et al., 2001). These challenges for cattle manure restrict the ability to efficiently process the manure with AD.

#### 2.4 Assessing Anaerobic Biodegradability

The main biogases produced from AD of cattle manure are methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>). The methane produced has several uses, the most relevant one currently being renewable energy. Methane can be used directly as a fuel for electricity generation, heating, and combustion in engines to produce mechanical power. In 2019, the California Public Utilities Commission issued the implementation of Senate Bill 1440. The Senate Bill established targets for renewable natural gas purchases by California's gas utilities. By 2030 California utilities will be required to supply 12% of renewable natural gas. California is representing the future of renewable natural gas and precedent that could soon be followed by other states. The utilization of biogas from cattle manure using AD can help reduce the large amount of greenhouse gas emissions. Biogas production helps mitigate odors and other potential environmental impacts associated with manure storage management (Nishio and Nakashimada, 2007).

#### 2.5 Biochemical Methane Potential Test

The BMP test can be beneficial for different types of organic substrates to characterize the ultimate methane production. In 2006, the Association of German Engineers published the first version to outline a guide for BMP testing known as VDI 4630 titled "Fermentation of Organic Materials". VDI 4630 gave the baseline terms and guidelines for BMP test and future protocols and was a factor for the batch assay protocol. To have valid BMP results the following parameters have to be met (Holliger et al. 2016).

- All tested samples must be tested with at least triplicated BMP bottles.

- There must be positive controls (e.g., cellulose, tributyrin, or microcrystalline) and negative controls (no substrate is added).
- The duration of the BMP test is not fixed. The test should be terminated when the amount of methane produced over a three-day span is less than 1% of the total accumulated volume of methane for each sample.
- The BMP volume data should be expressed as dry methane gas under standard conditions per mass of VS added.

## CHAPTER 3: MATERIALS AND METHODS

The objective of this study was to determine characteristics of cattle manure based on day durations of the manure left outside, and to see if changes in seasons had any effect on BMP results. For each collection, the manure sampled from the CAFO was left within the cattle pens for a specified amount of time, which ranged from 7 to 90 days, which will be referred to as manure accumulation periods.

### 3.1 Beef Feedlot Manure Management

Cattle manure used for this study was collected from a CAFO in the intermountain west region.

The typical manure removal process used by the feedlot consists of pens being cleaned out approximately every 60 days. First, a front loader enters the pen with a box blade that helps break up the manure pack while leaving a few inches of the manure pack on the surface. Manure is then placed into a pile, typically in the back of the pen. The pile is then collected out of the pen and transported to composting windrows.

### 3.2 Experiment Setup

The manure samples collected for this study were gathered in a different process than the normal method the feedlot uses in the removal of manure from the pens (Section 3.1). Four sample collections occurred in four different months which were in May 2022, August 2022, November 2022, and April 2023. Manure accumulation periods can be seen in Table 1.

Table 1: Manure accumulation periods for sample collections represented by X.

Manure Accumulation Period	Sample Collections			
	May 2022	August 2022	November 2022	April 2023
7 Day	X	X	X	
14 Day	X	X	X	
21 Day		X	X	
28 Day	X			
48 Day		X	X	X
56 Day	X			
90 Day			X	

The manure left in the pens before collection was exposed to different weather conditions over all four seasons. Each sample collection had 1 to 3 pens worth of manure from each desired manure accumulation period. Manure from each pen for each manure accumulation period was processed and tested for %TS and %VS and BMP as described in the following sections.

### 3.2.1 Temperature and Precipitation

As stated above, the sample collection occurred during different seasons. The pens from which manure was collected were outdoor and uncovered. The manure collected was subject to the seasonal changes including temperature and precipitation. The temperature and precipitation data were collected from the National Centers for Environmental Information from a station near the CAFO location.

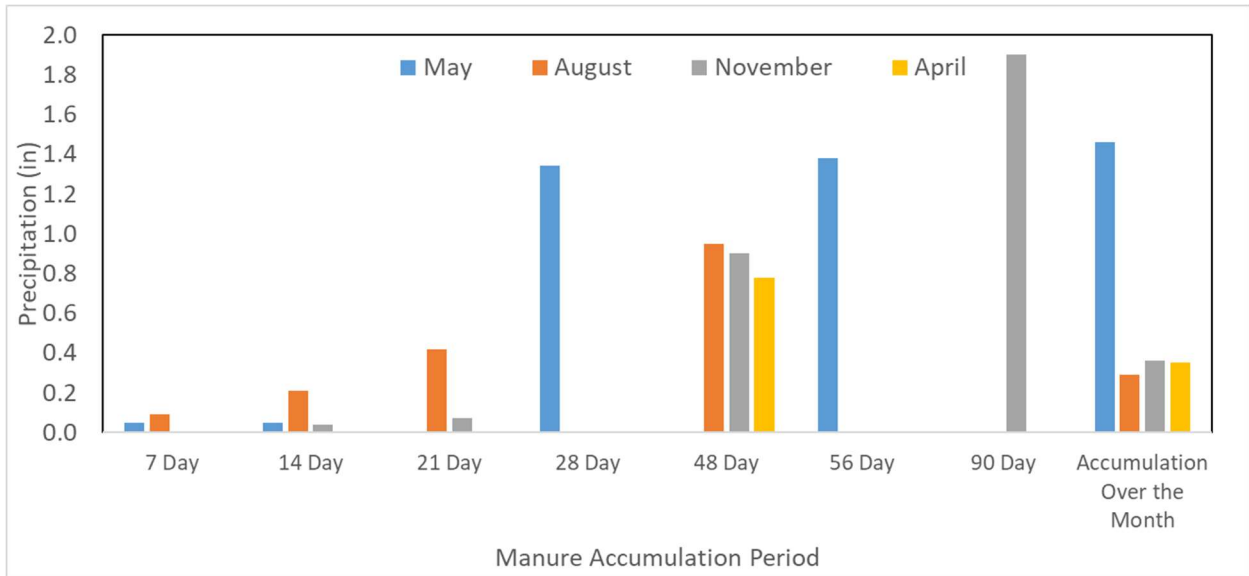


Figure 2: Precipitation reported accumulated over period (e.g., precipitation for 7 day is the sum over the 7-day period) and for the sample collection months.

For each manure accumulation period, shown in Figure 2, the precipitation is the total amount over the duration that the manure was in the pen. As day durations increase the amount of precipitation increases.

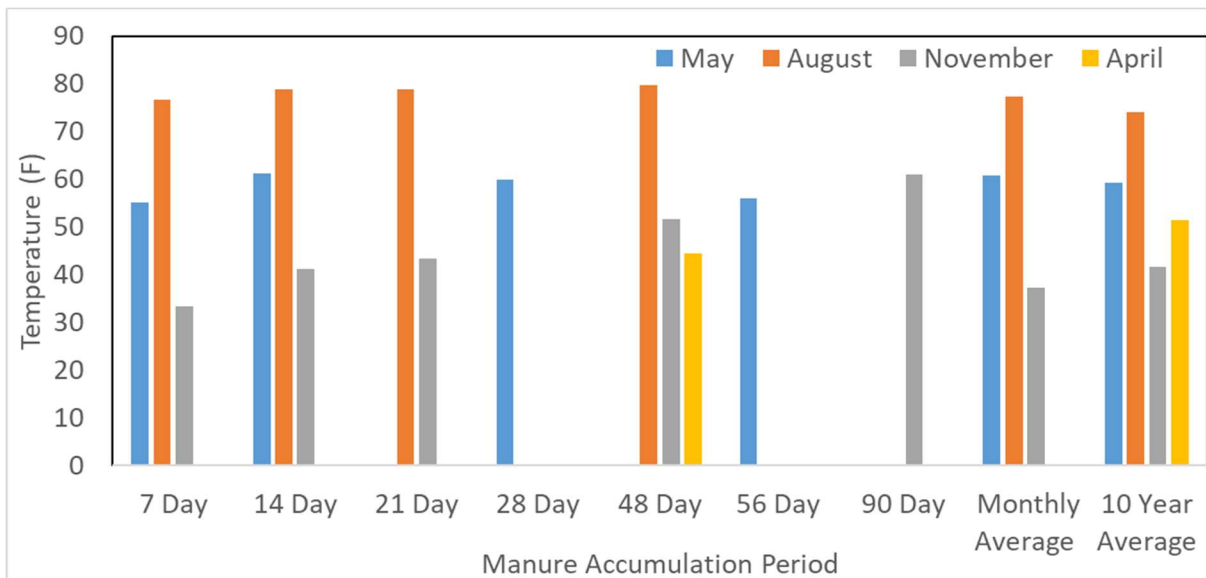
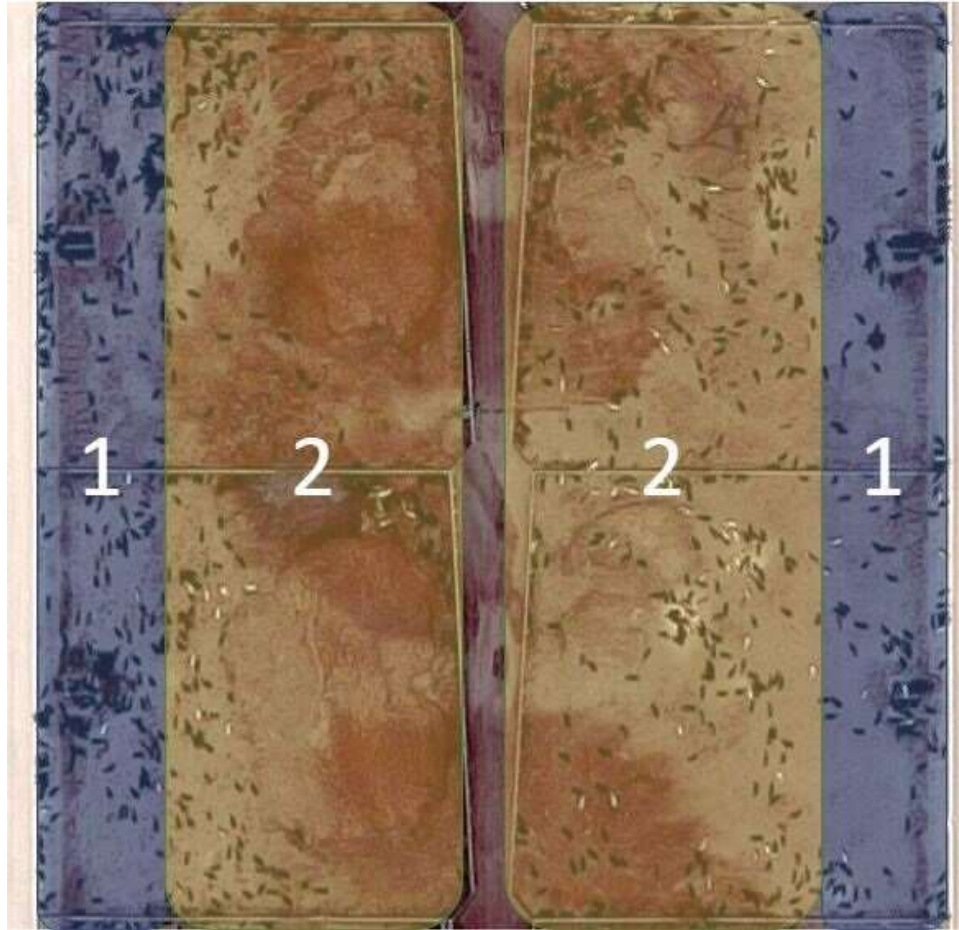


Figure 3 reported are an average over the periods reported (e.g., temperature for 7 day is a 7-day average). Monthly averages and the 10-year average are reported on the right side.

For each manure accumulation period, shown in Figure 3, the temperature was an average temperature determined over the manure accumulation period the in the pen.

### 3.3 Manure Collection and Preparation

Cattle manure used for this study was collected from a CAFO in the intermountain west region. For each manure accumulation period, three pens were selected based on similar types of feed and the total number of cattle. The May sample collection included one pen of manure for each manure accumulation period. Manure from piles collected from each pen was broken up into two different zones, zone 1 and zone 2 (Figure 4). Zone 1 was the outer portion of the pen and zone 2 was the inner portion of the pen. The goal with this method was to see if the manure collected in different locations of the pen was variable in quality and assess feasibility of targeting one zone for more frequent collection events. The BMP results from the May sample collections and an image representing the zones can be seen in Appendix A. However, due to this approach not showing a notable difference between zones (Appendix A) and was not a feasible method for long term collection, the other three sample collections did not include zones and manure from 3 pens was collected for manure accumulation period. For the remaining three sample collections each manure accumulation period included collection of manure from three pens.



*Figure 4: Zone 1 and Zone 2 in a singular pen*

For each sampled pen, the manure was scraped into a pile, loaded out, and taken to the weight station (Section 3.3.1). After the manure was weighed for each pen, it was placed into a singular row. For each pen, two 3.5-gallon buckets were used to collect a representative sample of the total mass. However, based on data and total weights of manure coming from each pen, a challenge occurred when collecting lower manure accumulation periods. Due to the manure pad not being perfectly leveled it was harder to only obtain the lower day manure accumulation periods with no manure pad being scraped. To try to have a better understanding of the lower day manure accumulation periods a second manure collection technique was adapted. The second method consists of land surveying one pen base elevations and collection manure on a 10 and 20-

day manure accumulation period based on the change of elevation (Section 3.3.2). For each manure accumulation period three points in the pen where elevation difference was shown, manure was collected. The collected manure from both methods was then grinded, divided, and sieved (Section 3.3.3). The sample was then placed into a freezer and stored at a temperature of 0 degrees Fahrenheit for future analysis. The freezer allowed the manure to keep the initial moisture content to capture the manure's characteristics as collected.

### 3.3.1 Manure Collection at from Pens

For the sample collection process, to gather the manure, a scraping method was conducted which was slightly different than the typical feedlot manure removal process described previously. Before the cattle were placed into the pen, the feedlot's crew cleaned out all of the pens. This scraping method consisted of a front-end loader pushing the manure into a pile in the back of the pen with no box blade attached (Figure 5). Due to the pens not being concrete and only having a manure pack on the bottom, the box blade was not necessary for the lower manure accumulation periods of manure collected. If the box blade was used, it would have added much older manure into the test sample which would affect the BMP results.



*Figure 5: Front-end loader with no box blade*

Once each pile was collected for all manure accumulation periods within a sample collection date, then the manure was placed into loading trucks. Based on the manure accumulation period, the number of truck loads varied with total mass. Each truck was weighed both before manure was placed into it and again after. The manure from each truck for each pen was placed into a singular row (Figure 6). To obtain the representation sample, 10 flag stakes were placed at equal distances around the row. At each flag, the first 6 inches deep into the pile was removed due to weather from manure sitting in direct sunlight for 1-6 hours. Then one shovel amount from each flag below the 6 inches was placed into both 3.5 L buckets.



*Figure 6: A singular row of manure from one pen with flag stakes placed for sample collection.*

### 3.3.2 Land Surveying Lower Manure Accumulation Periods for Manure Samples

A pen was land surveyed in April using an electronic theodolite after being scraped just before the cattle were placed into the pen (Figure 7). For each 10- and 20-day manure accumulation period, six random points within the pen were measured for base elevation. Only three points were collected although six were initially measured for base elevation as a safety precaution. The location of each point was noted and measured from two sides of the pen by using a 300 ft tape measure. On days 10 and 20, a 3.5-gallon bucket was collected for each point based on the elevation change and was collected within a 3 m radius of the point. After the sample was collected, the sample went through the same preparation process as loader collected samples (Sections 3.3.3).



*Figure 7: Selected land surveying pen initial conditions in April 2023*

### 3.3.3 Sample Preparation

As stated previously there were three pens selected for each manure accumulation period and a homogenous sample was collected. To create a composite sample for each manure accumulation period from all three pens of manure collected, rigorous sample processing was conducted. The sample preparation process consisted of grinding the manure, sample dividing the manure, and sieving the manure through a 3/8" sieve. The purpose of this process was to have a representative sample of the manure collected that as best as possible represents that manure accumulation period.

#### *Sample Grinding:*

For the BMP analysis using the batch assay, the manure particle size was a key parameter for BMP testing. Larger particles take longer to degrade in the first step of AD, hydrolysis. Smaller

particle sizes can degrade faster and achieve more accurate BMP data in a shorter time period. The particle size of the substrate is fundamental for kinetic studies rather than for BMP determination (Sanders2001; Vavilin 2005) but does impact duration of the BMP test. To reduce the manure size for efficient BMP determination, the manure collected first was processed through a mechanical grinder [JWC Environmental Model 10000 Muffin Monster]. As stated previously, 2 buckets were collected for each pen, the purpose of which is to have the first bucket go through the grinder to clean out any lingering material, while the second bucket for that pen will be placed into the grinder. The material from the first bucket was discarded and the material from the second bucket was the material that continued to being processed.

#### *Sample Dividing and Sieving:*

The second bucket of sample material that was processed through the grinder was carefully placed in Humboldt Riffle-Type Sample Splitter (H-3989). The sample splitter divides the sample into smaller portion sizes while keeping a representative sample. Each sample was processed through the sample divider three times, until sample size volume filled half of the liter bag. The sample from the sample divider was placed into a 1-gallon aluminum mylar vacuum sealed bag. The mylar bags were used to store samples to prevent water loss and reduce the amount of oxygen interacting with the sample. The other portion of the sample from the sample divider was placed back into the bucket and placed in a freezer.

Next, the sample was manually forced through a 3/8" sieve. The sieve was placed on top of a black bin to collect the sample after it passed through the sieve. After the sample was placed on top of the sieve, the sieve was gently shaken up and down to get most of the sample through the sieve. A portion of the sample was too large to go through the sieve. To allow that portion to fit, the sample was chopped by using sharp edge items. This allowed the sample to maintain

representability of the total sample. After all of the sample successfully was passed through the 3/8" sieve, it was placed back into the mylar bag and into the freezer for future use.

#### 3.3.4 Selection of Representative Samples for Manure Accumulation Periods

The sieved manure was analyzed for manure characteristics. First, the manure was sample divided through quartering method. The quartering method is when material is placed into a pile and separated into 4 sections from the middle of the pile to each side. One section will be picked to sample. The quartering method reduces the quantity of material while keeping a representative sample. A portion of the sample divide was used to determine %TS and %VS (Sections 3.4.1.1 and 3.4.1.2). The composite samples tested were a combination of manure collected from three different pens. The amount of manure from each pen added to the composited sample was determined by the TS contribution from each pen manure collected (as determined by %TS measurements, see Section 3.8) TS was used to generate a representative sample because it represented the total amount of dry manure from the contributing pens. Each pen manure varied in water content in the manure due to weather impact or the CAFO practices. In warmer conditions, the CAFO would have sprinklers on to cool off the cattle and keep the manure from becoming dust, thus. Composite based on wet mass may be subject to variability. The manure compositing process occurred for all sample collections and all analysis, including BMP, was conducted on these composited samples.

### 3.4 Analytical Methods

#### 3.4.1 Solids Characterization

The manure from the same manure accumulation period and collection day were combined based on a mass weighted average. Each dish was labeled using a number and letter punch set (6mm)

and heated at 550 degrees Celsius in an electric furnace (Fisher Isotemp 10-550-14 Benchtop Laboratory Muffle Furnace) for 30 minutes. After the dish was heated, the empty dish was weighed, and sample was added. Each dish was then placed in the furnace a second time and was taken out when the weight of each dish was not varying over a period of time (approximately 4 hours). Each dish was weighed, and mass weight was recorded upon removal from the oven.

#### 3.4.1.1 Total Solids

The percentage of total solids (%TS) was measured accordingly by using Method 1684 provided by the EPA. %TS represents the mass of solid material (or dry matter) that remains after removing the moisture from the sample. Samples collected directly from the manure piles that were not preprocessed as described in Sections X-X were placed in the pre-cooked aluminum pans, with a mass of 90-170 g. A mass of 15-40 g of the homogenized manure sample from each pen was sample divided, and the composited manure samples were placed in the pre-cooked aluminum dishes. The mass of the dish before and after placing the manure sample was recorded. The dish was then placed inside an electric oven to dry at 105 degrees Celsius until the weight stabilized (approx. 10-12 hours). The final mass of the dish was recorded, which represents the sample mass after 105 Celsius plus the pan weight.

$$\%TS = \frac{DM}{WM} * 100 = \frac{W_{total} - W_{dish}}{W_{sample} - W_{dish}} * 100$$

Equation 1: Percent Total Solids

$W_{dish}$  = Weight of dish (g)

$W_{sample}$  = Weight of wet sample and dish (g)

$W_{total}$  = Weight of dried residue and dish (g)

DM (dry mass) is the total weight of the sample with no water content, otherwise known as TS.

WM (wet mass) is the total weight of the sample with water content.

#### 3.4.1.2 Volatile Solids

The procedure of determining the percent volatile solids (%VS) for each sample was provided in Method 1684 by the EPA (Solids Sampling Methods). VS represents the mass of total solids that volatilize at 550 degrees Celsius. The dish from the TS contained the manure sample from each pen that was sample divided and the composited manure for the manure accumulation periods were placed in the 550 C furnace to determine the amount of %VS present in each sample. The dish was kept in the furnace until the weight of the dish stabilized (approximately 4 hours). The final mass of the dish was then recorded. The equation used to determine the percent VS can be seen below (equation 2):

$$\%VS = \frac{VM}{DM} * 100 = \frac{W_{total} - W_{ash}}{W_{total} - W_{dish}} * 100$$

Equation 2: Percent Volatile Solids

$W_{dish}$  = *Weight of dish (g)*

$W_{total}$  = *Weight of dried residue and dish (g)*

$W_{ash}$  = *Weight of residue and dish after ignition (g)*

VM (volatile mass), portion that volatilizes and burns off in the furnace, known as the organic portion.

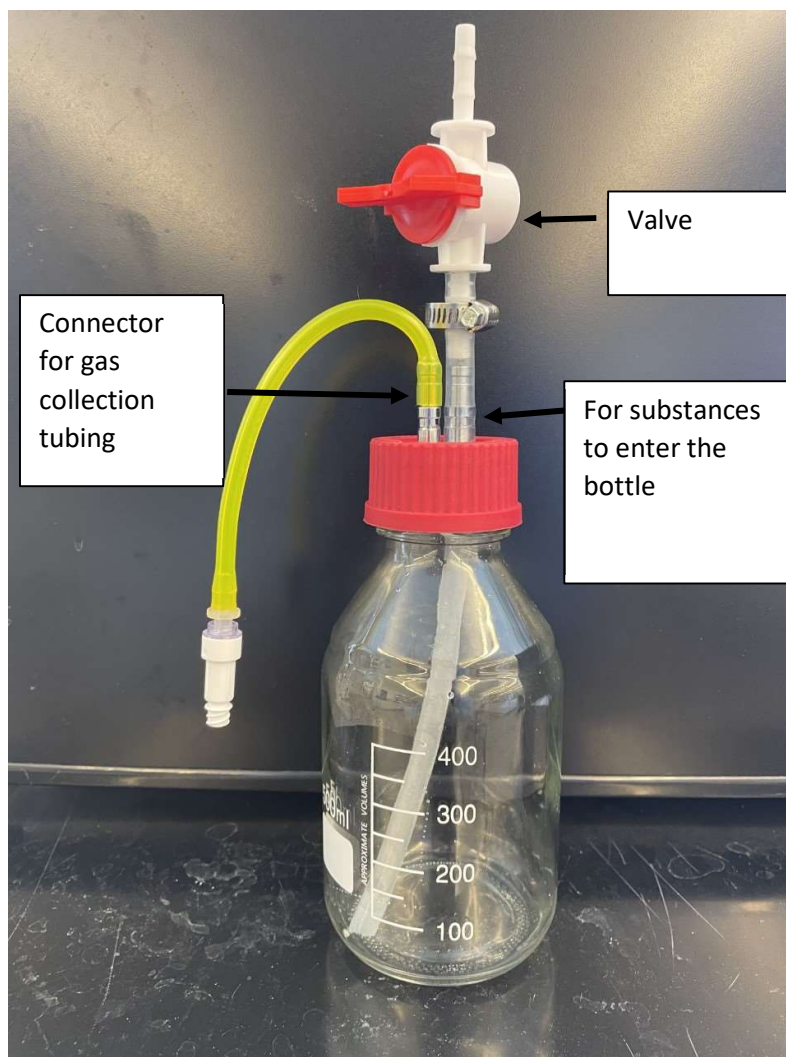
DM (dry mass) is the total weight of the sample with no water content, otherwise known as TS.

#### 3.5 BMP Batch Test

The manure collected for each manure accumulation period were composited based on a mass weighted average. The manure accumulation periods were then tested using batch assays for BMP. Each batch assay bottle consisted of inoculum and nutrient medium to allow for the

degradation process seen in Section 3.6. For each manure accumulation period, composite samples were tested in triplicate BMP bottles placed into an orbital shaker (Fisher Orbit Incubator Shaker, model 3525). The protocol for batch assays stated the importance of having at least three replicates for each sample. The replicates allow for statistical analysis to determine the reproducibility of the assays (Angelidaki et al. 2009). The duration of the BMP test is not a fixed amount of time: the test should be terminated when the production of methane gas over three consecutive day is less than 1% of the cumulative volume of methane produced for all BMP bottles being tested (Holliger et al. 2016).

For each manure accumulation period of each collection, triplicate BMP bottles were operated and acted as anaerobic digestors. For every BMP run for each sample collection, there were triplicate BMP bottles for both the positive and negative controls. The negative control bottles contained *only* the inoculum and nutrient medium; this allows information to be gathered about how much methane is produced from just the inoculum. The positive control bottles contained inoculum, nutrient medium, and microcrystalline cellulose with 95% of VS (super fine). Each bottle has a volume of 500 mL with an airtight headpiece to maintain anaerobic conditions. The head consists of a metal circular plate with two-cylindrical metal connectors along with an outer head to screw onto the bottle. Resin was used to create a stronger seal around the metal top opening to reduce the leakage of gases. On one of the cylindrical metal connectors, tubing is used to connect a valve (red and white) to allow the inoculum and nutrient medium enter the bottle (Figure 8). The second connector is used for the gas produced in the bottle to travel into the gas collection bottle.



*Figure 8: BMP bottle setup including the red valve and tubing that will connect to a gas bag.*

Before the sample is placed inside the bottle, each bottle is tested for leakage via a defined protocol (Appendix C). Once the bottle passes the leakage test, the gas collection bag is added, and a tubing bag leakage test was conducted (Appendix D). For the bottle, tubing, and bag to pass the leakage tests, allowing them to be used for BMP measurements, they must have a leakage rate of less than 0.5% per day.

After the sample amount was added to each bottle, the bottles were immediately flushed with nitrogen gas. For the flushing process, the BMP cart has two tubing connections, both connected to flow rate monitors. The bottle stayed connected for 3 minutes at a flow rate of 0.5 L/ minute.

### 3.6 Inoculum & Nutrient Medium

Inoculum is added to BMP test bottles to provide several different microorganisms which are crucial for accelerating the degradation process. The inoculum is required to be *fresh*- meaning it came from an active anaerobic reactor. The inoculum should also be degassed, meaning it had been pre-incubated to deplete the residual biodegradable organic material (Angelidaki et al. 2009). A relatively small amount of inoculum is often desired due to the potential production of biogas that can affect results. On the other hand, too little inoculum used can lead to process overload caused by acidification. It is recommended to use inoculum collected from anaerobic sludge from wastewater or to use inoculum derived from digestate of agricultural plants treating manure as the main feedstock (Holliger et al. 2016). The inoculum used for this research project was a digestate with the main feedstock being dairy manure.

Nutrient medium is required for some solid substrates that are micronutrient deficient such as cow manure. Nutrient medium is associated with the inoculum and is added during degradation (Angelidaki et al. 2004). The nutrient medium consists of many minerals and a low composition of organic carbons (Angelidaki et al., 2004). The nutrient medium consists of five mixed stock solutions, and the nutrient medium is created and placed into a container that is flushed with nitrogen. The amounts of each of the compounds in the stock solutions are listed below:

Anaerobic Basic Nutrient Medium consisted of 5 stock solutions.

Stock Solution A

- 100 g/L of  $NH_4Cl$
- 10 g/L of  $NaCl$
- 10 g/L of  $MgCl_2 \cdot 6H_2O$
- 5 g/L of  $CaCl_2 \cdot 2H_2O$

Stock Solution B

- 200 g/L of  $K_2HPO_4 \cdot 3H_2O$

Stock Solution C

- 0.5 g/L of Resazurin

Stock Solution D

- 2 g/L of  $FeCl_2 \cdot 4H_2O$
- 0.05 g/L of  $H_3BO_3$
- 0.05 g/L of  $ZnCl_2$
- 0.038 g/L of  $CuCl_2 \cdot 2H_2O$
- 0.05 g/L of  $MnCl_2 \cdot 4H_2O$
- 0.05 g/L of  $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$
- 0.05 g/L of  $AlCl_3$
- 0.092 g/L of  $NiCl_2 \cdot 6H_2O$
- 0.5 g/L of ethylenediaminetetraacetate
- 1 ml of concentrated  $HCl$
- 0.1 g/L of  $Na_2SeO_3 \cdot 5H_2O$

Stock Solution E

- 2 g/L of Biotin
- 2 g/L of Folic acid

- 10 g/L of pyridoxine acid
- 5 g/L of riboflavin
- 5 g/L of thiamine hydrochloride
- 0.1 g/L of cyanocobalamin
- 5 g/L of nicotinic acid
- 5 g/L of *p* – aminobenzoic acid
- 5 g/L of lipoic acid
- 5 g/L of DL – pantothenic acid

After each stock solution was created, 10 mL of stock solution of A was placed into a container. Then 2 mL of stock solution B and 1 mL of stock solution C, D, and E was added. Lastly, 974 mL of distilled water was added and mixed.

### 3.6.1 BMP Cart Setup

The BMP cart was setup to for anaerobic addition of inoculum and nutrient medium, with subsequent nitrogen flushing. It consists of two steel cylinders: from the front, the left side contains the inoculum, and the right cylinder consists of the nutrient medium (Figure 9).

Between the two cylinders, there are three valves connected with the tubing. The left valve connects the inoculum cylinder to a syringe when turned to the right. When the left valve is turned to the right and the middle valve is turned to the left, then the inoculum will be transferred to the middle valve that connects to the BMP bottles. The right valve connects to the nutrient medium cylinder, when valve is facing to the right then the nutrient medium will fill the syringe attached to the tubing. When the right valve is facing to the left and the middle valve is facing to the right, the nutrient medium can be transferred to the BMP bottles. The inoculum is always inserted first into each of the BMP bottles due to thicker consistency of the solution and a small

portion remaining in the middle valve tubing line. The amount of inoculum added to each bottle was 375 mL (75% of the bottle's total volume). The nutrient medium is the second component added to the BMP bottles. The nutrient medium flushed the remaining inoculum out of the line into the BMP bottles. It is important for the inoculum to stay at a temperature of 36 degrees Celsius to keep the microorganisms active.



*Figure 9: BMP Cart setup where BMP bottles were configured in an anaerobic system. The left container contained the inoculum and the right container contained nutrient medium.*

When adding the inoculum and nutrient medium, the valve connected to the long tubing needs to be open to allow for the release of nitrogen gas with replacement of that volume being replaced with the inoculum and nutrient medium. On top of each bottle there is a red valve that connects to the tubing from the BMP cart, controlled by the middle valve. The tubing that connects to the

BMP bottle has the same red valve, and when the inoculum and medium are added to the BMP bottle, both red valves must be open. After the inoculum and medium are added to the BMP bottle, it is necessary to close all valves that are attached to the bottle.

### 3.7 Orbital Shaker

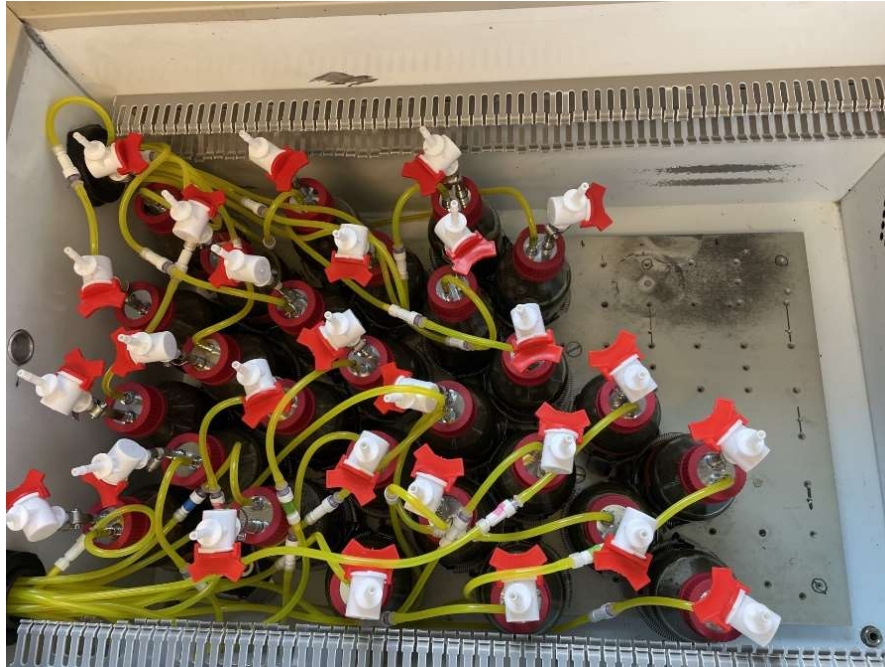
Each BMP bottle was inserted into individual flask bottle holders (2 3/4" diameter). The second connector on the BMP bottle was attached to a long tube that allowed produced biogas to flow into a gas collection bag, located on the outside of the orbital shaker (Figure 10). The gas bag used was a 1-liter foil bag from CEL Scientific Corp, suitable for samples with a low molecular weight. A three-way valve is used to connect the syringe to the gas bag, the valve is where gas from the bag can be taken out and tested. It is recommended to only fill the bag to a maximum volume of 800 mL.



*Figure 10: Gas collection bags located outside the orbital shaker.*

The orbital shaker can hold up to 45 BMP bottles and holds a temperature of 35 degrees Celsius.

The orbital shaker has a metal plate where the flask bottle holders are screwed into that that rotates constantly at a speed of 25 rpm (Figure 11).



*Figure 11: BMP bottle configuration inside orbital shaker with tubing exiting on the left to gas collection bags.*

### 3.8 Determination of Sample Quantity for BMP Bottles

The amount of manure for each manure accumulation period collection varied based on the %VS of the inoculum and the selected inoculum to substrate ratio (ISR). The ISR is a crucial factor in AD, it refers to the proportion of inoculum (material that starts the AD process) to the substrate (material that will be digested). The ISR is the ratio of VS of the inoculum to the VS of the manure by mass (equation 3).

$$ISR = \frac{g \text{ VS of Inoculum}}{g \text{ VS of Substrate}}$$

Equation 3: ISR

The %VS of the inoculum was measured 1-2 days before each batch test assay started. After the %VS of inoculum was determined the VS mass in grams of inoculum was determined by

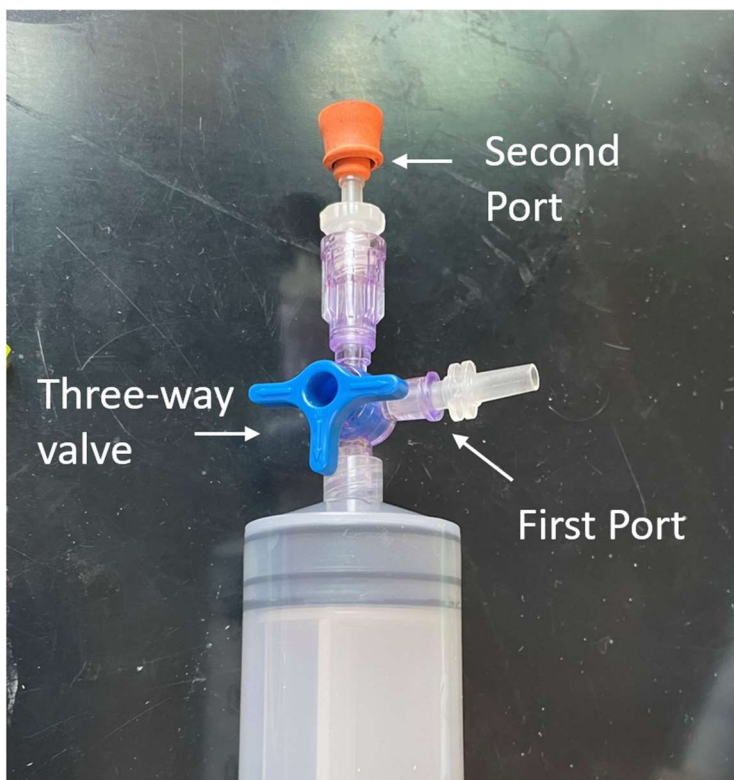
converting the %VS inoculum concentration to grams per liter. Based on the grams per liter concentration of inoculum it was then multiplied by the volume of inoculum in the BMP bottle. The VS from the inoculum should be greater than the VS from the substrate to minimize acidification. The selected ISR for each batch test assay was 1.5 for all manure accumulation periods and 3 for the positive controls. The selected ISR and the mass of inoculum VS were used to determine the mass VS of substrate to be added of each sample.

### 3.9 Gas Collection and Testing

Every 3-4 days for each batch BMP test, a 100 mL syringe was used to abstract gas from each of the gas bags. For the first three days of each batch BMP test, the gas volume was relatively low. The following 7 days after there would be an exponential increase in the amount of gas for each sample. The 100 mL syringe tip has a luer lock that is connected to a three-way-valve (Figure 12). The valve has a connector on the first port to obtain gas from the bags. The gas bag had tubing attached from the bag to the BMP bottle with a three-way valve in the middle. The three-way-valve was located approximately 5 inches away from the gas bag outside the orbital shaker. The first port was attached to the three-way-valve on gas bag tubing. After the three-way-valve was attached to the gas bag it was turned to only allow gas to flow *out* of the bag into the syringe. The syringe three-way valve was opened to only allow gas to flow from the bag *into* the syringe. The second port that is in line with the syringe has a female fitting with a rubber septum stopper (Figure 12). The syringe would be filled anywhere from 1-9 times depending on what point the gas collection part of the test was at.

Based on the outside appearance of the gas bag, it was relatively simple to see the relative amounts of gas in the bag. If there was little gas (e.g., less than 100 mL) only 20 mL was initially collected, both three-way valves were then closed, and the first port was disconnected. The

syringe three-way-valve was turned to allow the gas to flow from the syringe to the second port to flush the rubber septum. If the gas bag had a lot of gas (e.g., between 100- 800 mL), then 100 mL was initially collected to flush the second port. After the second port was flushed the process repeated until all gas from the gas bag was collected. The gas collected between the initial collection and final syringe collection from the same bag was flushed through the second port. The final syringe is pulled from the bag, and the total volume is recorded (including the flushed volume). The gas in the syringe is inserted into a Gas Chromatograph (GC) to determine the percentage of methane produced for that sample. For each bottle in the orbital shaker, a syringe with at least 5mL of gas was tested through the GC.



*Figure 12: 100 mL syringe luer lock tip with three-way-valve.*

### *Methane Gas Analysis*

A well-defined protocol was used for operation of the GC (Appendix E). The GC used for this study was an HP 6890 Series GC System with a Rt-QS-Bond Plot column (30m, 0.32 mm ID, 10  $\mu\text{m}$ ). Before the gas from the syringes were tested, the GC was calibrated by using pure methane gas (Ultra High Purity Grade Methane, Size 300 High Pressure Steel Cylinder, CGA 350) purchased from Airgas. For the calibration process, 100, 80, 60, and 20  $\mu\text{L}$  samples were inserted into the GC. A linear calibration curve is created to estimate methane concentration of tested samples.

From each BMP batch test assay, the gas collection syringe was then punctured with a 100  $\mu\text{L}$  needle syringe (Hamilton 100 ul Syringe 81038) in the second port. The needle syringe was then used to pull a 100  $\mu\text{L}$  sample out from the gas collection syringe. The first 100  $\mu\text{L}$  was then flushed out of the needle syringe. The process of obtaining the gas was repeated and then inserted into the GC three times. For each injection the methane peak was recorded. The average of all three methane peaks for each bottle was averaged and the calibration curve was used to estimate percentage. For each BMP bottle, the quality control criteria were to have lower than a 5% standard deviation from the three methane peaks.

#### 3.10 Normalizing Cumulative Methane Production

The methane volume yield was expressed as the cumulative volume of methane gas under standard temperature and pressure (STP-273 K, 1000 hPa) per mass of volatile solids added to each BMP bottle. The biogas yield of a substrate is highly dependent on the content of organic

compound which degrades under anaerobic conditions (VDI 4630). The following steps were used to properly express the methane production:

#### Step 1: Determining Methane Gas Volume Produced

- Methane volume for each BMP bottle was determined by multiplying the total vented volume (mL) by the average methane %.

#### Step 2: Normalizing Methane Gas Volume to STP

- The methane volume was then converted to STP (Equation 4)

$$\text{Equation 4: } V_{STP} = \frac{P_{gas}}{P_{STP}} * \frac{T_{STP}}{T_{gas}} * V_{gas}$$

Pressure units: hPa

Temperature units: Kelvin

T<sub>stp</sub>= 273 K

P<sub>stp</sub>= 1000 hPa

#### Step 3: Subtracting Gas Production of the Inoculum from the Sample Gas Production

- The methane volume produced from each BMP bottle contained methane production from the substrate and the inoculum. To determine the total volume of methane produced from the substrate, the total volume of methane produced in the BMP bottle was calculated by subtracting the gas production by the inoculum (i.e., 3 negative controls) (Holliger et al. 2016).

#### Step 4: Normalize Gas Production to methane mL CH<sub>4</sub>/ g VS

- The methane volume determined in step 3 was divided by the grams of VS of substrate added based on the target ISR (Section 3.7).

#### Step 5: Cumulative Methane Volume Production

- The BMP production from each time point was summed to estimate cumulative methane volume produced.

#### 3.11 Assessing Differences in Seasons and Manure Accumulation Periods

To gain a better understanding of the differences in methane production for different samples (seasons and manure accumulation periods), multiple statistical analyses were conducted including an analysis of variance (ANOVA), pairwise, and a T-Test. For this set of data, ANOVA was used to determine if the data compared was significantly different or not. However, the ANOVA test could not be used on all data comparison tests due to different sample size sets, which is why the pairwise and T-Tests were then conducted. If the P-value determined in the stated statistical analyses was greater than the significance level (0.05) then the data compared is not significantly different. If the P-value is less than the significance level, then the data compared is significantly different.

## Chapter 4. Results and Discussion

### 4.1 Characterization of Solids in Manure Samples

For each sample collection, the %TS and %VS were tested for each manure accumulation period using the methods described in Sections 3.4.1.1 and 3.4.1.2.

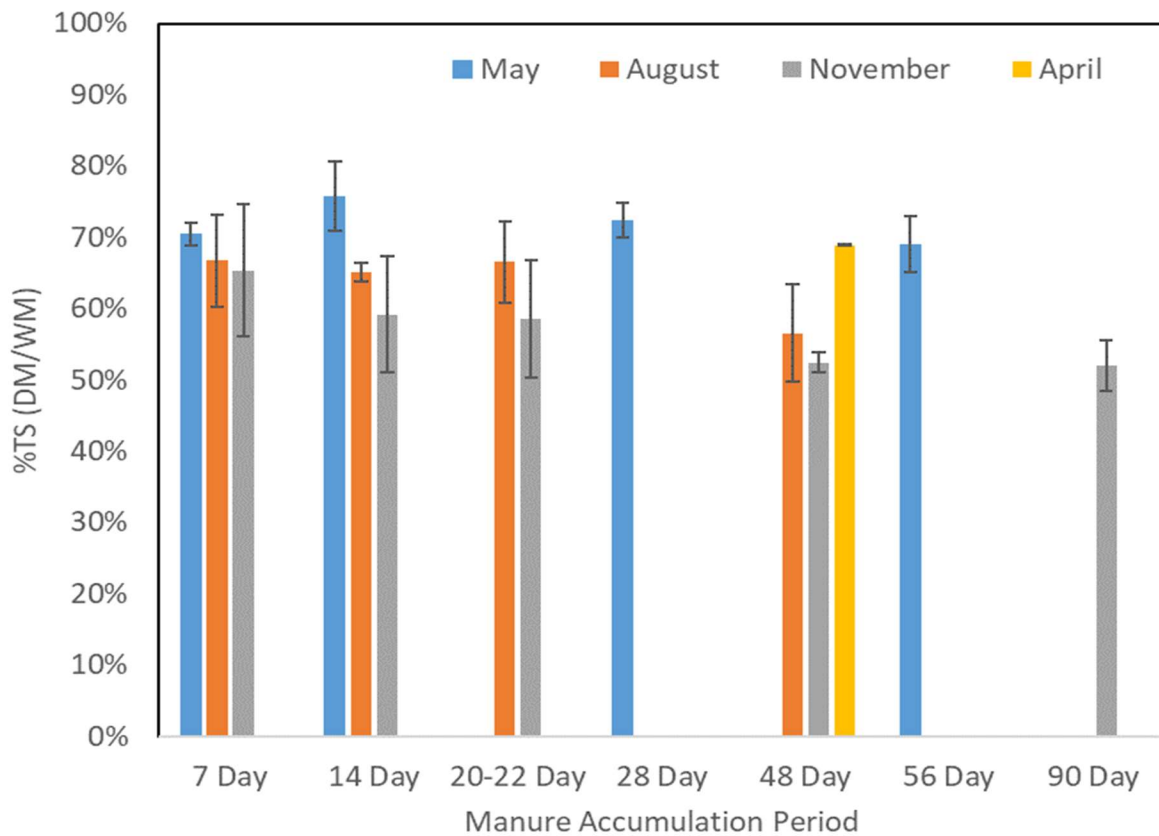


Figure 13: %TS reported for all four sample collections for each collected manure accumulation period. Error bars indicate  $\pm$  one standard deviation (includes three pens and three technical replicates).

It would be expected for %TS to increase with manure accumulation period if manure was not affected by precipitation. For the August collection, the %TS is relatively the same for the first three manure accumulation periods (7-day, 14-day, and 20-22-day) and decreases by 10% TS for the 48-day manure accumulation period. However, when manure was collected during warmer months in the intermountain west, the CAFO turned on sprinklers to cool off the beef cattle in pens. The sprinklers did impact the %TS of manure collect creating a barrier to compare manure from different seasons with represent to %TS. For the fall months, during cooler weather conditions, the %TS was expected to stay the same due to minimal moisture content evaporating (i.e. lower temperature; see Figure 3), however, the November sample collection had a decrease in %TS with increase in manure accumulation period. A similar phenomenon can be seen for the winter seasons, however, as the intermountain west tends to get snow and more precipitation in winter compared to other seasons which would therefore increase the moisture content. The spring season would have caused results similar to what was observed in the fall if the manure was covered and did not interact with precipitation. Another factor that could have affected the %TS of the sample collections was the collection method of manure and how over scraping could potentially happen.

Provided from the temperature data, the sample collection with the warmest temperature was the August sample collection, the coolest temperature sample collection was November. With August having higher temperatures then November sample collection it would be expected that the %TS for the August sample collection would be higher than Novembers. With can be slightly seen in Figure 13.

Based on Figure 13 the %TS from the manure collected from each sample collection there was no observable trend between %TS and the duration of manure accumulation periods. The total

cumulative amount of precipitation for each manure accumulation period. From the precipitation data, the May sample collection had more precipitation compared to the other sample collection around the 56-day manure period. This is common in the intermountain west due to snowmelt and related spring conditions (Figure 2, Section 3.2.1). Based on Figure 2 it would be expected the longer manure accumulation periods would have higher water content. However, seasonal conditions affect the water content in the manure. This could have been affected by the CAFO sprinklers during warmer months causing the water content not to be accurate of seasonal conditions. In conclusion, the %TS determined in this study did not show a correlation with seasonal impacts.

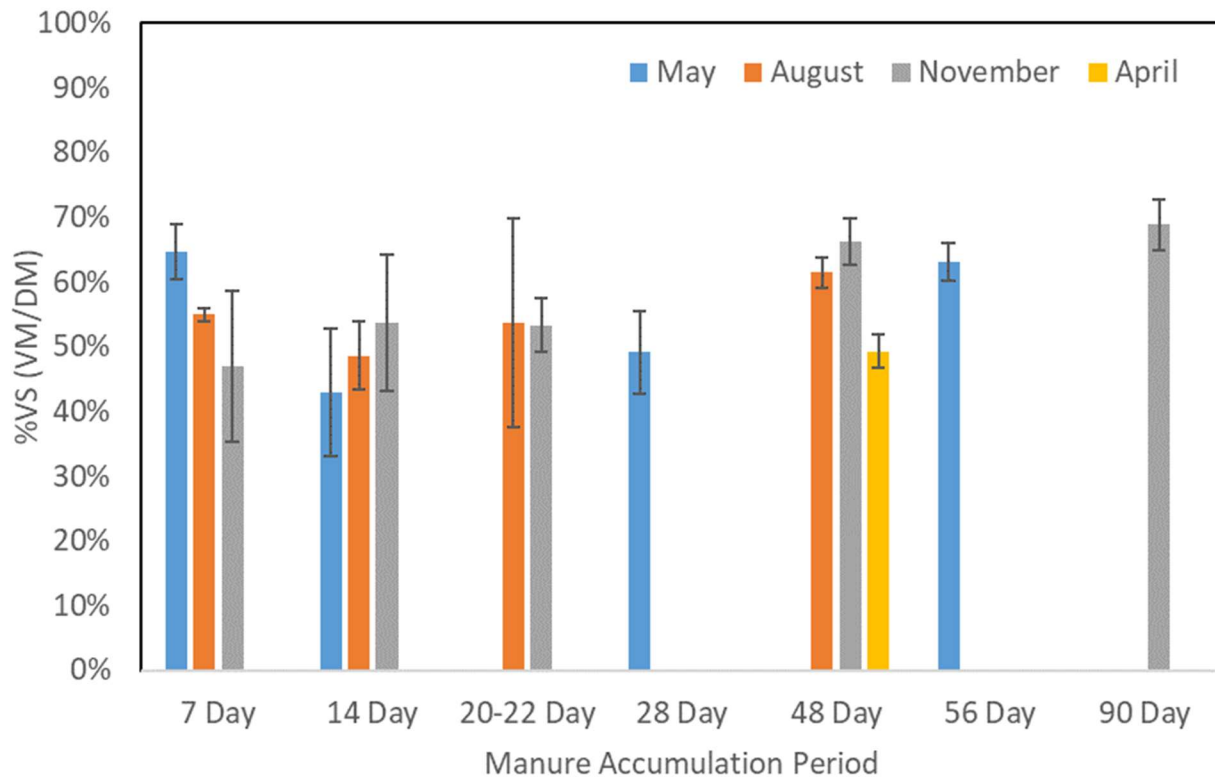
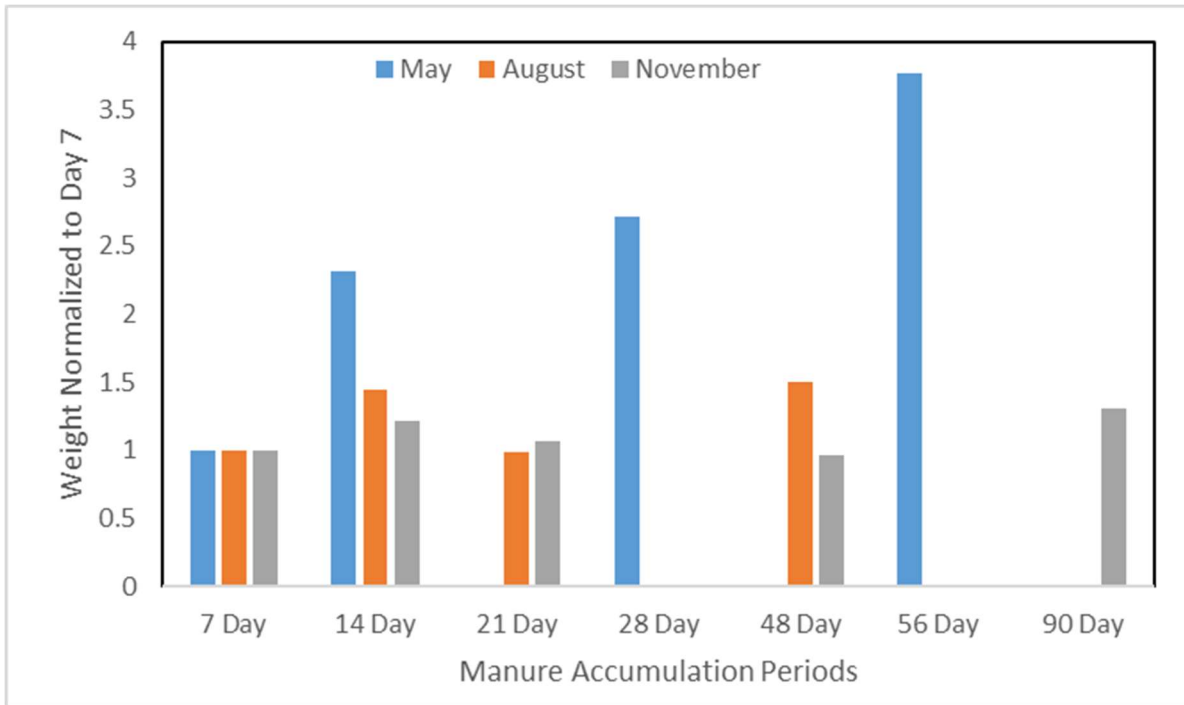


Figure 14: %VS reported for all four sample collections for each collected manure accumulation period. Error bars indicate  $\pm$  one standard deviation (includes three pens and three technical replicates)

It would be expected for %VS to decrease with the manure accumulation due to biodegradation of organic solids. For all four sample collections there was no consistent, observable decreasing trend of %VS with duration of accumulation periods (Figure 14). It is important to remember with the collection method that over-scraping into the packed manure was a concern based on visual observation of manure collection and the collected samples. The measured %VS, and lack of decrease in %VS with accumulation period, confirmed the visual observation that over scraping was occurring for the lower duration manure accumulation periods. This would affect %VS and %TS of the sample due to the manure pack being exponentially older material. Based on Figure 14 there were no notable trends between manure accumulation periods and sample collections.

#### 4.1.1 Normalized Sample Collection Total Weight

To assess the potential for scraping of the manure pad into samples collected during low accumulation periods, the mass of collected samples was evaluated. For each sample collection, the manure collected from all three pens for each manure accumulation period was weighed to determine total weight. The expected weight total should increase linearly with manure accumulation period.

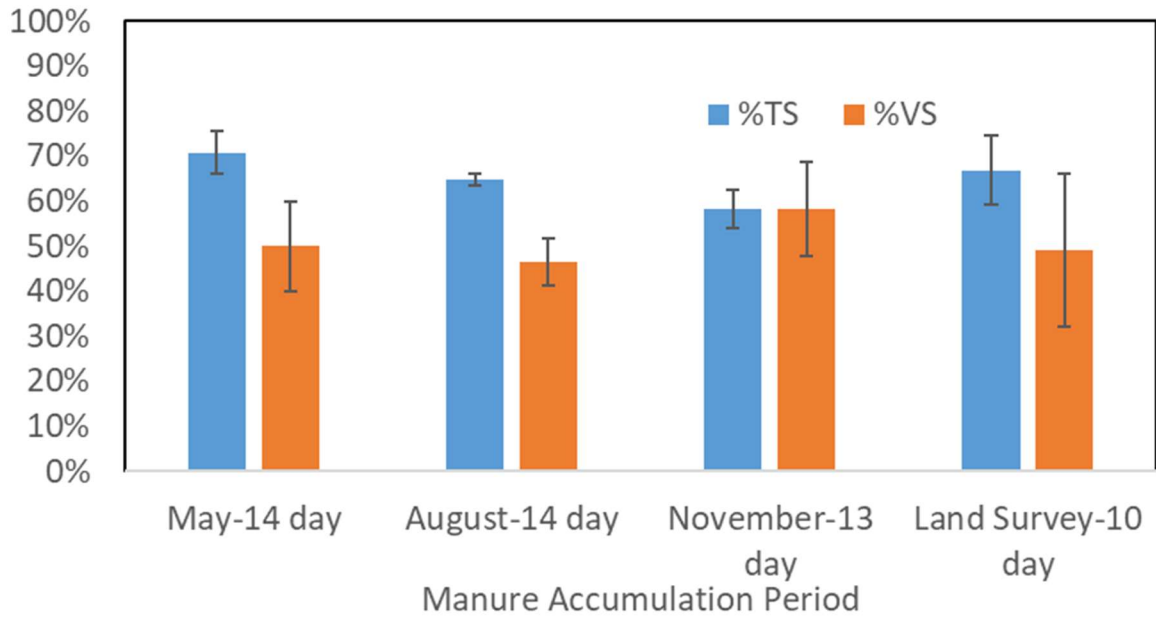


*Figure 15: Total weight of manure collected from each manure accumulation period, normalized to day 7 weight. April sample collection is not included due to it not having a 7-day manure accumulation period.*

Figure 15 represents the total weight for each manure accumulation period normalized to the 7-day manure weight. Based on the normalization to the 7-day data the 14-day data hypothetically should be double the amount of the 7-day data. The May sample collection had a trend of increasing manure accumulation periods with total weight, although not entirely linear (the 56-accumulation period should be near 8 times higher than day 7 but is much lower than that). The August and November sample collections did not exhibit the same trend. One would expect to see the 14-day manure collected be double the amount of the 7-day manure collected. The manure weight normalized to the 7-day manure collection along with % VS trends (Figure 14) demonstrate difficulty in collection of samples that are only fresh for each accumulation period.

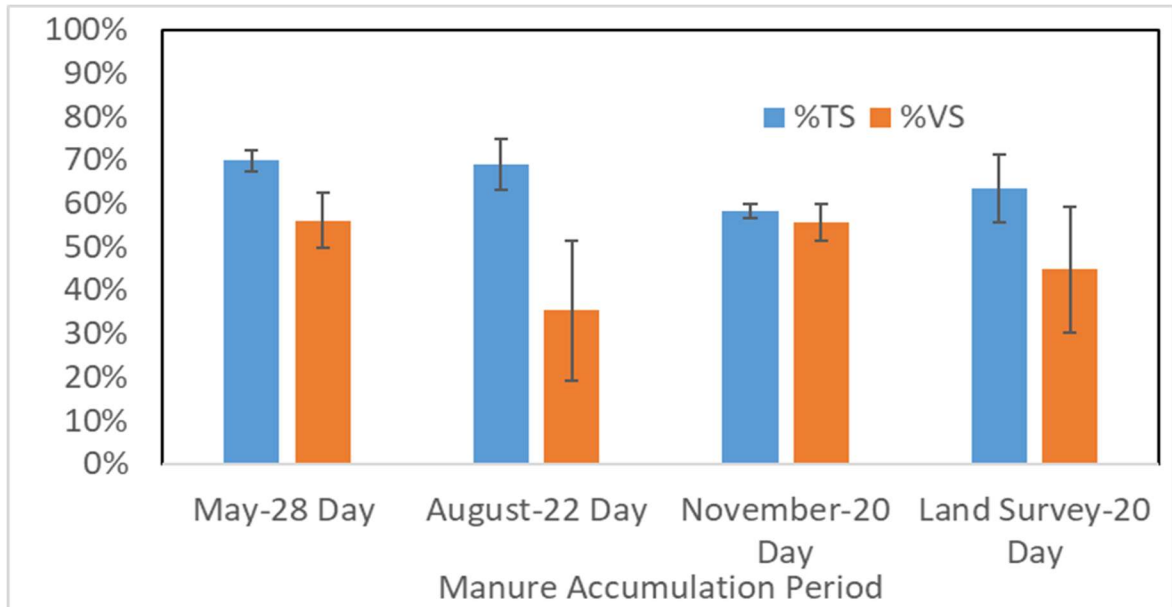
#### 4.1.2 Total Solids and Volatile Solids of the Manure Collected by the Land Surveying Method

The %VS and %TS were tested for the manure collected by land surveying manure collected.



*Figure 16: %TS and %VS for 10-day land surveyed manure compared to similar manure accumulation periods. Error bars indicate  $\pm$  one standard deviation (includes three pens and three technical replicates).*

The 10-day land surveying manure %TS was comparable to the manure accumulation periods (13-14 days) for each sampling event. However, the %VS was similar to the 14-day August sample collection and slightly lower than the May and November sample collections, but with no notable differences present between the land surveying method (10-day manure accumulation period) and the sample collections in terms of TS and VS (Figure 16).



*Figure 17: %TS and %VS for 20-day land surveyed manure compared to similar manure accumulation periods. Error bars indicate  $\pm$  one standard deviation (includes three pens and three technical replicates).*

Similar, to the 10-day land survey data, the 20-day land survey data was compared to the sample collections from the manure accumulation periods that were as close as possible in manure accumulation duration (Figure 17). Under ideal laboratory conditions and identical seasonal conditions, the 10- and 20-day land surveying manure hypothetically should have had higher %VS, due to the different manure collection technique not obtaining the manure pad. However, due to the pre-selected points surveyed and collected, it did not ensure that there would be a layer of fresh manure (Gopalan et al., 2014). There were a few factors that affected the land surveying method, the first being weather conditions. When the initial elevations were taken, the pen was muddy, and the manure pack was not completely pressed down. When collecting the 10- and 20-manure accumulation periods, this led to elevation heights being the same, lower than the initial, or slightly higher than the initial elevation height. Another factor that potentially affected the elevation heights is that the manure pack is not perfectly flat, making it extremely important to measure the initial and future elevation heights as accurately as possible at the desired location

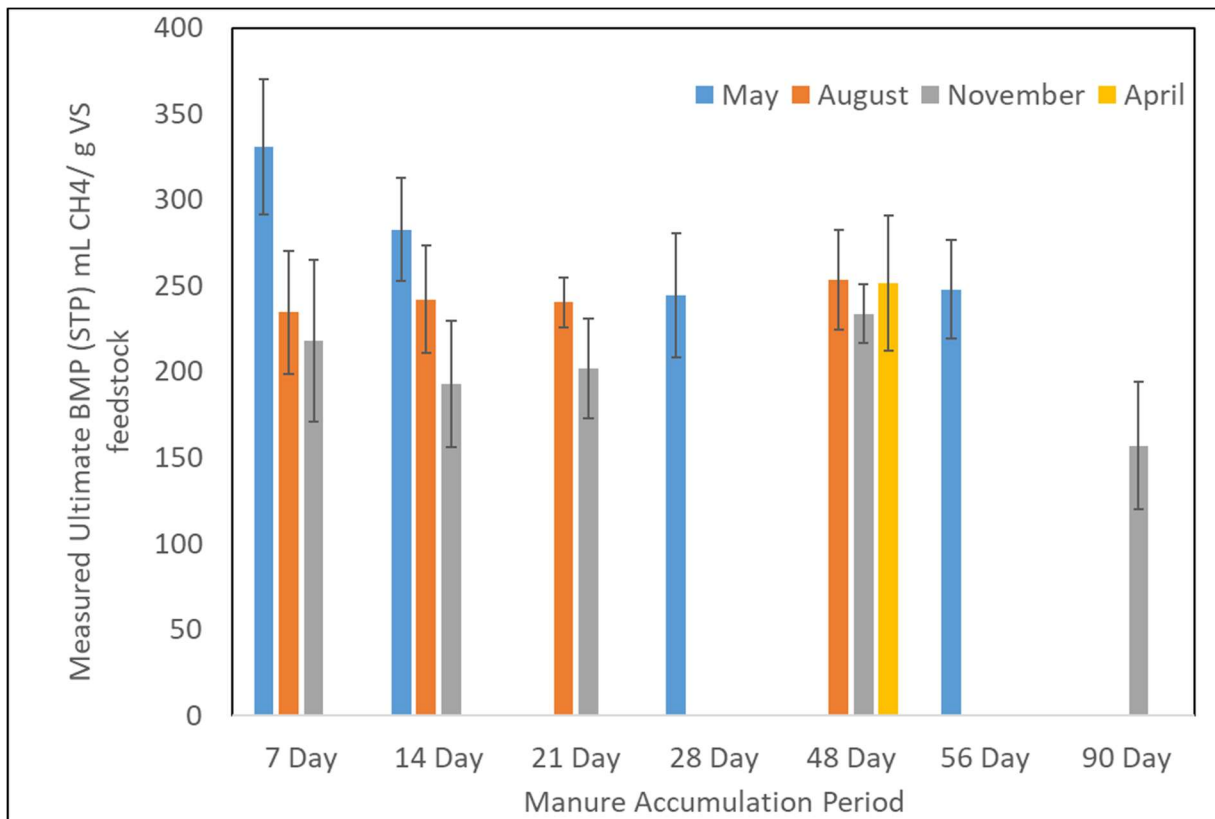
within the pen. The elevation difference could have been affected by factors like cattle digging into the liner or choosing to spend most of their time laying in certain spots, which has been explained by employees at the feedlot. One of the desired spots in the pen can be seen in Figure 18 representing the uneven surface. Consequently, there was potential that a portion of the manure pad was collected and mixed into the manure sample. Figure 17 shows no notable change between the 20-day land surveying manure compared to similar manure accumulation periods from sample collections. The complexity in manure characteristics and impacts from cattle tramping on manure make collection of fresh manure (only deposited after last sample collection) very difficult to achieve in practice.



*Figure 18: Land Survey desired spot representing uneven surface.*

## 4.2 BMP Batch Assay Results

The measured ultimate BMP data from the manure collections using the method in section 3.3.1 (not including the land surveying data) can be seen in Figure 19. The measured ultimate BMP data is normalized to VS (Section 3.10). May sample collection manure accumulation periods were tested in zones. In Figure 19 the average of the two zones for the May sample collection is shown for each manure accumulation period.



*Figure 19: BMP results with respect to VS for all four sample collections including all manure accumulation periods. Error bars indicated  $\pm$  one standard deviation (includes three BMP bottles and three technical replicates for methane and VS analysis).*

The May collection produced more methane than was produced during the other seasons (Figure 19). The May collection has a decreasing trend as the manure accumulation period increases, which is not the case for the other sample collections. One difference between the May sample

collection and the other collections was the more careful management of pens and scraping of the pens. The May collection had more of a detailed plan, precision with the scraping method, and more supervision from Colorado State University team/ feedlot staff. The other three sample collections followed the same format, but however did not have as much stressing the importance of not over scrapping. The trend in methane production for the May sample event was consistent with the observation of a trend in manure mass collected (Figure 15). This further suggests the difficulty in obtaining accurate samples that include only freshly deposited manure material. Data seen in Figure 19 represents the total weight of manure collected for each manure accumulation period normalized to 7-day manure weight. Figure 19 strengthens the idea that the May sample collection had a more precise manure collection process than the other sample collections. This could indicate the other two sample collections had an inconsistent amount of manure collection from each manure accumulation period. This would affect the BMP results due to the %VS being proportional to the total amount of manure pad was collected. Which potentially led to the May samples having the highest BMP data from this study. The over scrapping hindered this studies ability to assess impacts from seasonal conditions and manure accumulation periods (not including May sample collection) on BMP results.

Based on Figure 19, the 7-day manure accumulation period contained three of the sample collections (May, August, and November). Based on the temperature and precipitation data it would make sense that May would have the highest BMP data due to the manure being potentially preserved by cooled temperatures. The next highest BMP data for the 7-day manure accumulation period would expect to be the November manure collected due to November having cooled temperatures than August sample collection. However, Figure 19 represents May having the highest BMP results than August than November for the 7-day manure accumulation

period. Based on Figure 19 there were no observable trends difference between BMP results from the different seasons.

To further assess whether there may be an impact on BMP for manure accumulation duration to see if there were any trends that were not captured with BMP data normalized with respect to VS, BMP was also normalized to TS (Figure 20). This represents the methane production potential for the total material. However, based on Figure 19. There are similarities in the data trends for accumulation periods when normalized to VS (Figure 19) and TS (Figure 20). For example, the May sample shows the highest methane potential for an accumulation period of 7 days and decreases for higher accumulation periods while there is no observable difference in methane production for the August and November samples. There are no notable trends across seasons that are explainable.

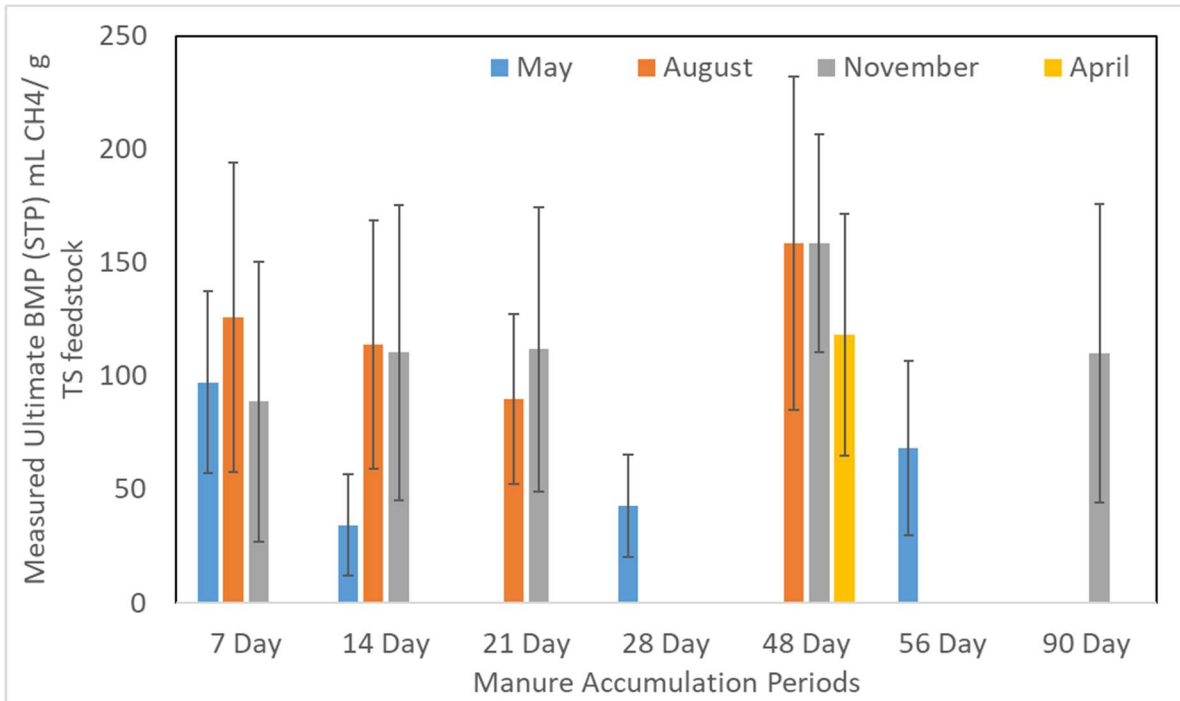
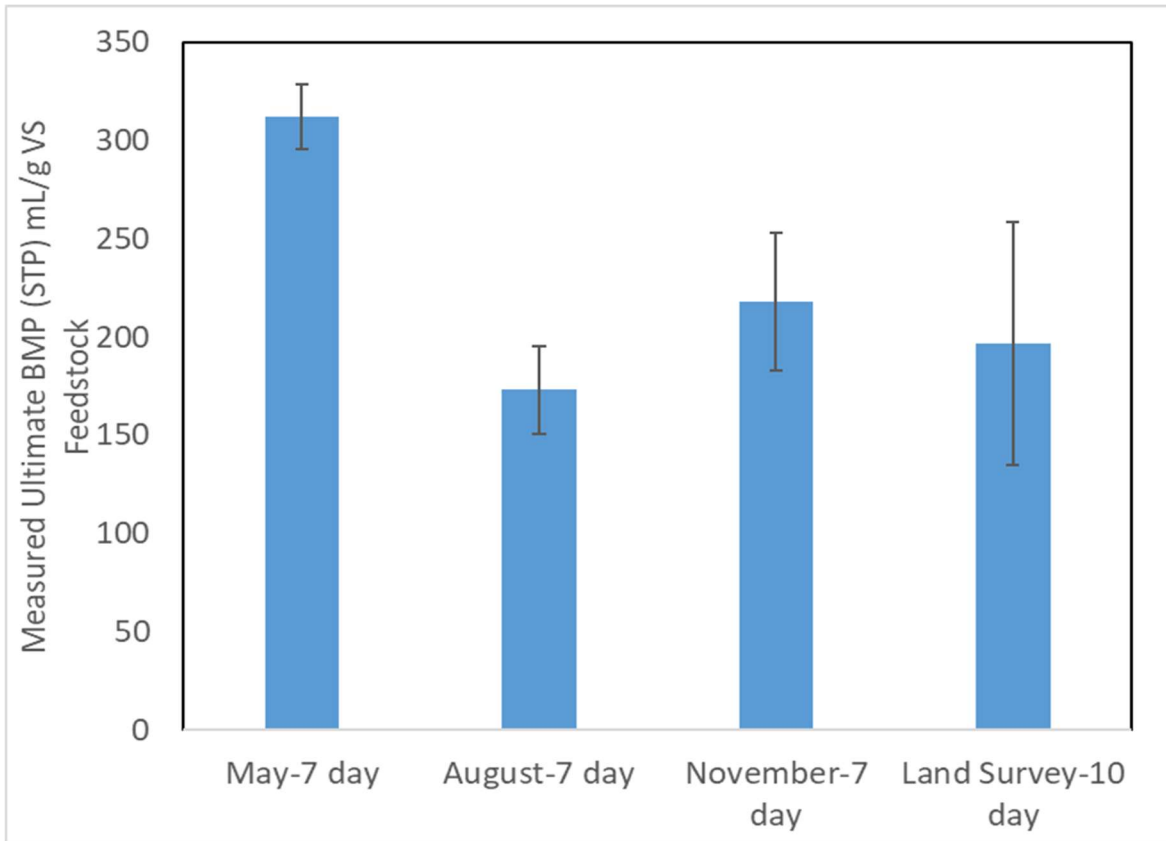


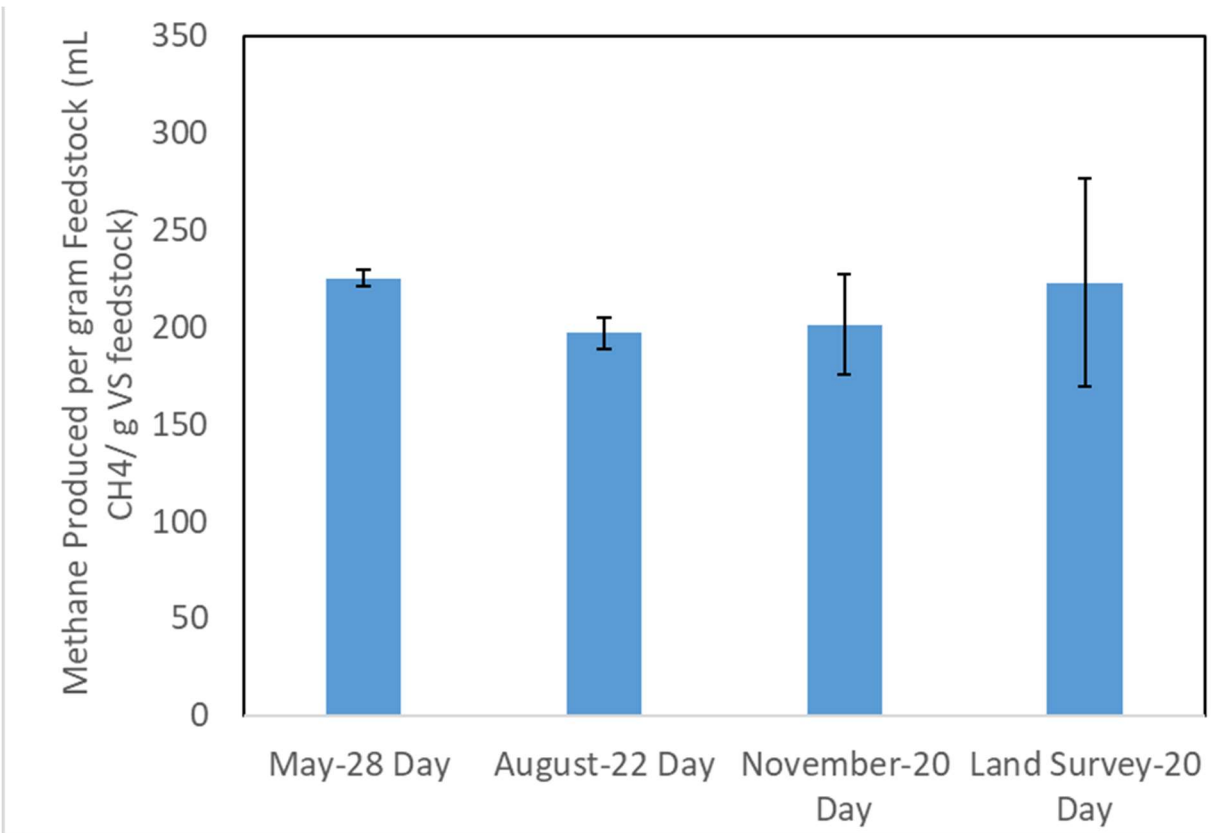
Figure 20: BMP results with respect to TS for all four sample collections including all manure accumulation periods. Error bars indicate  $\pm$  one standard deviation (includes three BMP bottles and three technical replicates for methane and VS analysis).

#### 4.2.1 Land Surveyed Samples Compared to Scraped Collection Samples

As summarized in Section 3.3.2, the land-surveyed manure collected was 10- and 20-day manure. Figure 19 represents the measured ultimate BMP of the 10-day land survey day compared to the first method of collection with similar manure accumulation periods.



*Figure 21: Measured Ultimate BMP comparison of the 10-day land surveying data to similar manure accumulation periods from sample collections. Error bars indicate  $\pm$  one standard deviation (includes three BMP bottles and three technical replicates for methane and VS analysis).*



*Figure 22: Measured Ultimate BMP comparison of the 10-day land surveying data to similar manure accumulation periods from sample collections. Error bars indicate  $\pm$  one standard deviation (includes three BMP bottles and three technical replicates for methane and VS analysis).*

Based on Figures 21 and 22, there is no observable difference between the land survey data and the scraped sample collections data. Stated in Section 4.1.2, there was a potential for a higher %VS for the land surveying data. However, the measured ultimate BMP would not change due to the ISR, but due to the amount of manure added to the BMP bottle being lower if %VS was higher. The land surveying method was adapted to obtain a more accurate collection of fresh manure for lower manure accumulation periods.

#### 4.2.2 Manure BMP Results for Accumulation Periods Compared to Literature

As stated previously, anaerobic digestion has been tested and used for decades on different types of organic materials. However, with the difficulty discussed with collection process, not as much research has been done on manure accumulation periods of beef cattle manure. Table 2 represents the average measured ultimate BMP for each manure accumulation period, including all sample collections.

*Table 2: Average BMP results from all sample collections by manure accumulation period.*

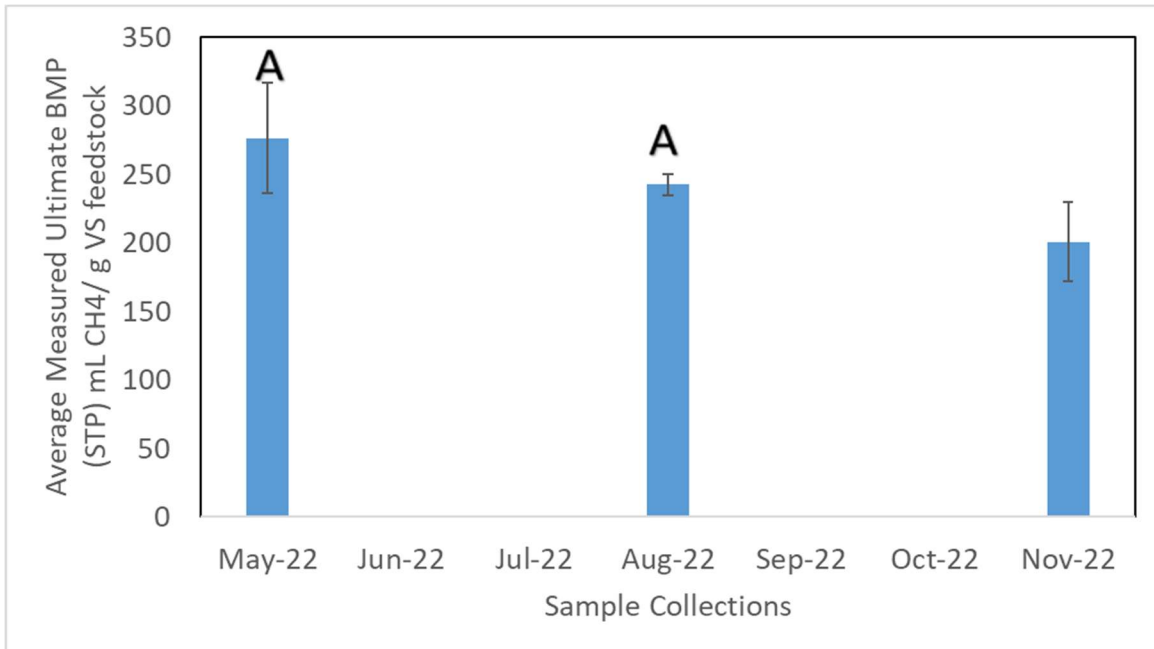
<b>Manure Accumulation Period</b>	<b>Average Measured Ultimate BMP (STP) mL CH<sub>4</sub>/ g VS feedstock</b>	<b>BMP Range</b>	<b>Sample Collection Included</b>
7 Day	261 ± 60.8	235-331	May, August, and November
14 Day	239 ± 44.9	193-283	May, August, and November
21 Day	221 ± 27.3	202-240	August and November
28 Day	244	-	May
48 Day	246 ± 10.9	234-253	August, November, and April
56 Day	248	-	May
90 Day	157	-	November

Another study compared different types of beef cattle manure which included fresh manure (less than 8 hours old), pad manure (manure accumulated from the last scraping), and stockpiled manure (8–12-month-old manure). The pad manure is the most comparable to the manure used for this study and the test was conducted at 37 degrees Celsius. For a 21-day manure accumulation period, the BMP reported was 200 mL CH<sub>4</sub>/ g VS feedstock from the pad manure

(Gopalan et al. 2014). The average measured ultimate BMP from this study, the 21-day manure accumulation period, was 221 mL CH<sub>4</sub>/ g VS feedstock, which is slightly higher, but comparable to the reported BMP value for the same manure accumulation period. In the same study, there was data on a 56-day manure accumulation period for the pad manure. The recorded BMP value was mL CH<sub>4</sub>/ g VS feedstock, which is lower than the 56-day manure accumulation period from this study\_(248 mL CH<sub>4</sub>/ g VS feedstock). Environmental conditions such as precipitation and temperature may have been a factor.

#### 4.3 Comparison of Seasonal Differences in Cattle Manure Quality for Methane Production

As stated previously, each sample collection occurred during different times of the year with different seasonal characteristics, for example different temperatures and humidity levels. The average measured ultimate BMP of each sample collection was determined based on all manure accumulation periods for each collection. An ANOVA test was conducted to compare the sample collections. Figure 23 represents the average of all four sample collections and the measured ultimate BMP.



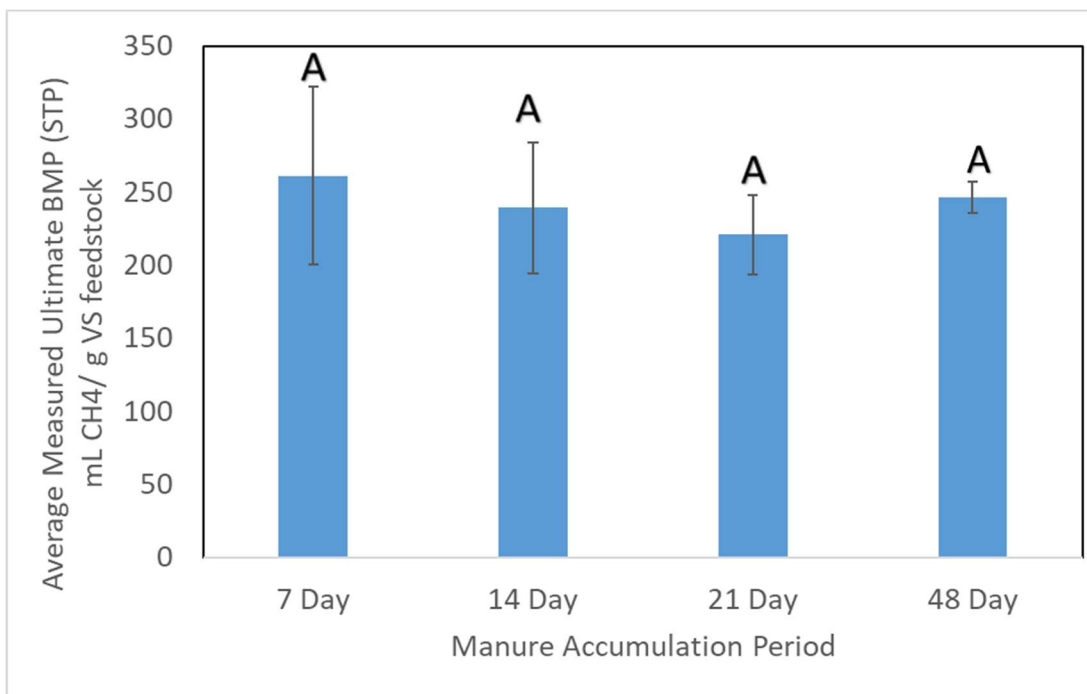
*Figure 23: August 2022 and May 2022 sample collection data, a symbol (A) was placed to indicate that there is no significant difference (P-value > 0.05). Error bars indicate  $\pm$  one standard deviation. May and August n is 4 (three technical replicates), November n is 5 (three technical replicates). April sample collection is not included on the figure due to only having one manure accumulation period.*

Above the August 2022 and May 2022 sample collection data, a symbol (A) was placed to indicate that there is no significant difference (P-value > 0.05). The manure collection for the May 2022, August 2022, and November 2022 sample collection had different amount of precipitation occur (Section 3.2.1). For the three sample collections also varied in temperature due to the collection time in season. The average temperature of the May sample collection for all manure accumulation periods was approximately 60 degrees Fahrenheit. The average temperature of the August sample collection for all manure accumulation periods was approximately 78 degrees Fahrenheit. The average temperature of the November sample collection for all manure accumulation periods was approximately 37 degrees Fahrenheit (Figure 3). With the November sample collection having the coolest temperature of the three sample collections seen on Figure 23, the November sample collection would be expected to have the

highest amount BMP data. Based on Figure 23 there was no observable impact of seasons that can be explained on BMP of the collected manure samples.

#### 4.3.1 Comparison of Manure Accumulation Periods for Sample Collections

An ANOVA test was conducted on average manure accumulation periods of the sample collections to determine if there was or was not any significant difference and therefore statistical significance.



*Figure 24: Statistical analysis on average sample collections manure accumulation period measure ultimate BMP. Error bars indicate  $\pm$  one standard deviation. The months included in each manure accumulation period varied as follows; 7- and 14-day manure accumulation periods (May, August, and November), 21-day manure accumulation period (August and November), and 48-day manure accumulation period (August, November, and April). The 28-, 56-, and 90-day manure accumulation periods are not included due to them only being in one sample collection.*

The 7-, 14-, 21- and 48-day manure accumulation periods contained at least two sample collections ultimate measured BMP results (Figure 24). From Figure 24 the ultimate measured BMP for the 7-, 14-, 21- and 48-day manure accumulation periods were the only periods that

were not significantly different ( $P\text{-value} > 0.05$ ) as represented by the “A” symbol. The other manure accumulation periods (28, 56, and 90 day) were not included in Figure 24 due to each manure accumulation period only having one sample collection manure.

#### 4.3.3 Pairwise Comparisons of Methane Potential

Table 4 includes all manure accumulation periods for all four sample collections on the x and y-axis. The content was determined by both pairwise and T-Tests to determine if the measured ultimate measured BMP was significantly different or not. The red squares represent a statistical difference between the two samples ( $P\text{-value} < 0.05$ ), green represents no statistically significant difference different ( $P\text{-value} > 0.05$ ), and the blank squares indicate a sample comparison to itself.

Table 3: Statical comparison of each manure accumulation period from sample collections where the duration of manure accumulation period is included in parentheses after the month of sample collection.

	May (7)0	May (14)	May (28)	May (56)	Aug (7)0	Aug (14)	Aug (21)	Aug (48)	Nov (7)0	Nov (14)	Nov (21)	Nov (48)	Nov (90)	April (48)
May (7)	R	R	R	R	R	R	R	R	R	R	R	R	R	R
May (14)	R	A	R	A	R	R	R	A	R	R	R	R	R	A
May (28)	R	R	A	A	A	A	A	A	A	R	R	A	R	A
May (56)	R	A	A	A	A	A	A	A	A	R	R	A	R	A
Aug. (7)	R	R	A	A	A	A	A	A	A	A	R	A	A	A
Aug. (14)	R	R	A	A	A	A	A	A	A	A	A	A	A	A
Aug. (21)	R	R	A	A	A	A	A	A	A	A	A	A	A	A
Aug. (48)	R	R	A	A	A	A	A	A	A	A	A	A	R	A
Nov. (7)	R	R	A	A	A	A	A	A	A	A	A	A	R	R
Nov. (14)	R	R	R	R	A	A	A	A	A	A	A	A	A	R
Nov. (21)	R	R	R	R	R	A	A	A	A	A	A	A	A	A
Nov. (48)	R	R	A	A	A	A	A	A	A	A	A	A	R	A
Nov. (90)	R	R	R	R	A	A	A	R	R	A	A	R	A	R
April (48)	R	A	A	A	A	A	A	A	R	R	A	A	R	

One clear observation from this comparison is that, when the lower manure accumulation periods of the May 2022 sample collection periods is compared to the other sample collections periods, it shows that the majority of the data is significantly different. In general, the May samples tend to show more difference when compared to other collected samples and May samples are also

different from each other. This demonstrates the difference in May samples that has been discussed above. This result was most likely due to careful management of the pens and manure collection for the samples (see Section 4.2). When the other three sample collections are compared (August 2022, November 2022, and April 2023) to one another, there is less significant difference than the May sample collection.

All of May samples compared with all other sample months are approximately 63% significantly different (Table 4). This demonstrated that May is the outlier. The August, November, and April samples compared to sample collections had a higher less than 40% significant difference (Table 4). This further demonstrates the difference for the May sample collection.

*Table 4: Represents % not significantly different and significantly different of each sample collection manure accumulation periods compared to each other seen in Table 4.*

<b>Sample Collection</b>	<b>Significantly Different from other</b>
<b>Month</b>	<b>Sample Collection Months (%)</b>
<b>May 2022</b>	63
<b>August 2022</b>	19
<b>November 2022</b>	38
<b>April 2023</b>	38

#### 4.4 Comparison of BMP Observed in this Study to Other Studies

Methane yield from beef cattle manure tested in this study varied between 157-331 mL CH<sub>4</sub>/ g VS feedstock. For the study, the highest methane yield was determined to be 331 mL CH<sub>4</sub>/ g VS feedstock from the May sample collection, 7-day manure accumulation period. The lowest methane yield was 157 mL CH<sub>4</sub>/ g VS feedstock from the November sample collection, 90-day manure accumulation period. Table 2 compares methane yield, %TS, and %VS from 14

researcher papers, compared to the average methane yield of each sample collection. All references in Table 5 use beef cattle manure, but it is important to note that the age of the manure was not stated in most of the papers, nor the collection method used.

Table 5: BMP results from this study compared to literature.

Reference	Material	Temp. (C)	%TS	%VS	BMP (mL CH <sub>4</sub> /g VS feedstock)	BMP Range	Article Type
This Study (Bhowmik 2023)	Beef Cattle Manure - May 2022	35	72	55	276 ± 33.5	248-331	-
This Study (Bhowmik 2023)	Beef Cattle Manure - August 2022	35	64	55	243 ± 27.7	235-253	-
This Study (Bhowmik 2023)	Beef Cattle Manure - November 2022	35	58	58	200 ± 33.4	157-234	-
This Study (Bhowmik 2023)	Beef Cattle Manure - April 2023	35	69	49	251	251 N/A	-
Hill et al. (1984)	Beef Cattle Manure	35	20	12.8	220-240	-	Peer reviewed
Moody et al. (2011)	Beef Cattle Manure	35	14.3	12.6	122.1	-	Peer reviewed
Moody et al. (2011)	Beef Cattle Manure (outdoor pen)	35	15.1	12.4	84	-	Peer reviewed
Wedwitschka et al. (2022)	Beef Cattle Manure (#1)	55	29	78	195	-	Peer reviewed
Wedwitschka et al. (2022)	Beef Cattle Manure (#2)	55	49.2	49.2	146	-	Peer reviewed
Wedwitschka et al. (2022)	Beef Cattle Manure (#3)	55	52.1	26.7	142	-	Peer reviewed
Guiling et al. (2020)	Cattle Manure	-	-	-	147	-	160 case studies
Varel et al. (1980)	Beef Cattle Manure	35	-	-	338	-	Peer reviewed
Ahring et al. (2001)	Beef Cattle Manure Diluted (4.5% VS)	55	-	-	202	-	Peer reviewed
Ahring et al. (2001)	Beef Cattle Manure Diluted (4.5% VS)	65	-	-	165	-	Peer reviewed

Wu-haan et al. (2010)	Beef Cattle Manure	35	29	24	120	-	Peer reviewed
Kaparaju et al. (2008)	Blended, Fresh Manure (diluted to 8%)	54	8.1	6.2	230	-	Peer reviewed
Buendia et al (2008)	Manure Mixed with Straw Bedding	-	31	20	86	-	Peer reviewed
Qiao et al. (2011)	Diluted Cow Manure	37	28	20	140	-	Peer reviewed

One of the references in Table 5 from researcher Guiling (2020) compared 160 case studies that used cattle manure in a mono-digestion. The highest methane yield based on Table 5 is the May sample collection conducted in this study resulting in an average BMP of 276 mL CH<sub>4</sub>/ g VS feedstock. Following right behind are the April and August sample collections from this study. The average BMP of the ten studies compared to was 160 mL CH<sub>4</sub>/ g VS feedstock. The last sample collection from this study is November, was higher than the average of the 10 other studies BMP results.

## Chapter 5. Discussion

In this study, four sample collections occurred in four different months during 2022 - 2023: May, August, November, and April. Each sample collection was analyzed for %TS, %VS, and BMP. The purpose of the study was to inform manure collection strategies and determine seasonal impacts on methane generation. No notable trends were observed for %VS and %TS from the sample collections for varying seasons or manure accumulation period duration. The manure collection process potentially over scrapped the pens, adding older manure (from manure pad) into each manure accumulation period manure collected. Causing difficulties in determining observable trends for %TS, %VS, and the BMP results. Another issue for %TS was during warm months in the intermountain west, sprinklers were on that added to the moisture content on the manure collected. Both the manure collection method and sprinklers where the potential cause was no observed trends within a majority of the data. For each sample collection, the manure accumulation periods were tested to determine the measured ultimate BMP. The May 2022 sample collection exhibited the highest measured ultimate BMP average among all manure accumulation periods, generating 276 mL of methane per gram of VS feedstock. In contrast, the other three sample collections had measured ultimate BMP averages ranging from 200 to 276 mL of methane per gram of VS feedstock.

Measured ultimate BMP is reported with relation to VS. In the case of the May sample collection, the measured ultimate BMP showed a linear decrease with increasing manure accumulation periods as would be expected. The %VS was the highest for the 7-day manure accumulation period for May, followed by a decline in the subsequent two periods, and then an increase in the final period. This resulted in no observable trend between %VS and BMP results for the May sample collection.

For the August sample collection, there was an increase in measured ultimate BMP with increasing manure accumulation periods, while %VS did not exhibit any noticeable trend with varying manure accumulation periods. A similar outcome was observed for the November sample collection. This was potentially due to the August and November sample collection manure collection method over scrapping the pens, especially with the lower duration manure accumulation periods. With over scrapping the manure pad was potentially collected and used for testing. Due to the manure pad being older manure it could have affected the %VS and BMP results.

Each manure accumulation period consisted of three pens worth of manure. The total weight of each pen was determined and combined for the total weight for each manure accumulation period. The weight was normalized to the 7-day manure accumulation period for each sample collection. The May sample collection showed a relatively constant trend with total mass of manure with increasing manure accumulation periods. However, the August and November sample collections had no trend between total mass of manure and increasing accumulation periods. The normalization of the weight made it easier to understand how the manure collection process is not easy to keep consistent based on the August and November sample collection.

A statistical analysis was conducted to examine differences between sample collection seasons, average manure accumulation periods, and all manure accumulation periods from each sample collection. The statistical analysis determined if the data compared was or was not significantly different. The August, November, and April sample collections manure accumulation periods were found not to be as significantly different then the May sample collection manure accumulation periods when directly compared (P-value > 0.05).

When comparing the average BMP data for each manure accumulation period (including all sample collections that contain that manure accumulation period), the 7,14-,21-, and 48-day manure accumulation period had no significant differences ( $P > 0.05$ ). However, the 28,56-, and 90-day manure accumulation periods were not part of this statistical analysis due to each manure accumulation period only having one sample collection.

The highest measured ultimate BMP value was from the May sample collection, despite displaying significant differences compared to the other sample collections in this study. This could be due to a more precise manure collection process. The findings in this study emphasize the importance of considering various factors and the potential influence on ultimate BMP results.

In response to potential discrepancies between the total weights of the pens (e.g., 56-day manure accumulation period should hypothetically have 8 times the weight of a 7-day manure collection period) collected during the sample collections and their corresponding manure accumulation periods and data, a land surveying method was employed to validate the accuracy of the sample collection technique. The land surveying method involved collecting manure from a single pen over 10- and 20-day accumulation periods in April 2023. However, the %VS, %TS, and ultimate measured BMP data obtained from the land surveying approach did not show any observational differences compared to sample collections with similar manure accumulation periods. This further points to complexity in manure collection methods that would enable collection of fresh manure only for the accumulation period.

Due to the challenges feedlots experienced with collecting lower day manure accumulation periods it will be difficult to only obtain the fresh manure without no manure pad. Beef cattle feedlots typically use a manure pad as the base on the pen, with this information there is not a

great method to only obtain the fresh manure. Typically, at the feedlot where manure was collected at, they tend to clean out manure from pens every 48 to 60 days. However, based on the BMP data there was no notable change for the August, November, and April sample collection between manure accumulations periods. Seen in BMP data from this study the May sample collection did show a significant difference (P-value < 0.05) compared to the other three sample collections. Based on the BMP results observed between the manure accumulation periods and the difficulty in collection of samples that included only freshly deposited manure, more frequent manure collection to enhance methane production does not appear to be highly promising or feasible. It may not be worthwhile to conduct further studies to collect lower day manure accumulation periods to assess quality due to typical management of manure collection at beef cattle feedlots.

To contextualize the measured ultimate BMP results from this study, a comparison was made with data from 14 other relevant studies. The data compared from this study was the sample collection manure accumulation periods and the measured ultimate BMP was averaged. The May sample collection exhibited the highest BMP value among all studies, followed by the April sample collection. The BMP values for the August and November sample collections were also relatively higher than those reported in the majority of the 14 case studies (Table 5).

## Chapter 6. Summary

From manure collected in this study in the sample collections BMP data varied between 157-331 mL CH<sub>4</sub>/ g VS feedstock. The highest methane yield was determined to be 331 mL CH<sub>4</sub>/ g VS feedstock from the 7-day manure accumulation period in the May sample collection. For each sample collection (May, August, November, and April) the average BMP results including all manure accumulation periods was compared to 14 other studies. The average BMP results from the 14 studies was 160 mL CH<sub>4</sub>/ g VS feedstock. The average BMP data from all four sample collections was higher than the average of the 14 studies. The data represented from this study is higher than other studies conducted indicating that beef cattle manure does have potential for producing higher BMP results than seen previously.

Having established that beef cattle manure has the potential to produce higher BMP results than previously observed, it crucial to understand what factors could have influenced the results from this study. This research study investigated the impact of manure accumulation periods and seasonal impact manure quality and methane production potential in the intermountain west. The study analyzed data and findings from four sample collections from different seasons. According to data and findings from this study, it was observed that manure collection process could have an effect ultimate BMP. The first sample collection which occurred in May was considered the most carefully managed. However, the subsequent three sample collections did not exhibit the same trend, prompting the exploration of an alternative manure collection technique that applied land surveying to attempt to collect only freshly deposited manure. However, the land surveying method encountered a similar issue that the sample collection method, which was with the collection of the manure pad.

Notably, the May sample collection had an observable trend between measured ultimate BMP and manure accumulation periods, demonstrating a decrease in ultimate BMP as manure accumulation period duration increased, which did align with expectations. The measured ultimate BMP values from all four sample collections surpassed the averages reported in previous studies from literature.

In conclusion, the study did not identify seasonal impacts through total solids (TS) data due to the CAFO typical operations (e.g., sprinklers). Establishing a direct relationship between TS to BMP results was hindered by the sample collection process. Results indicated that manure collected more frequently may produce more methane. However, manure sample collection by conventional methods and the land surveying method demonstrated complexity in the ability to collect manure that does not include the manure pad. For careful manure sample analysis, the ideal scenario for sample collection would involve a manure pad-free environment with a perfectly level and flat surface. However, the actual conditions deviated from this ideal, necessitating the recommendation for future studies to consider conducting small-scale experiments to gain more control over these types of variables. By doing this there could be more defined results on manure accumulation period and if seasonal conditions affect manure quality and characteristics. However, for beef cattle feedlot operations the lower day manure accumulation periods are very difficult to collect.

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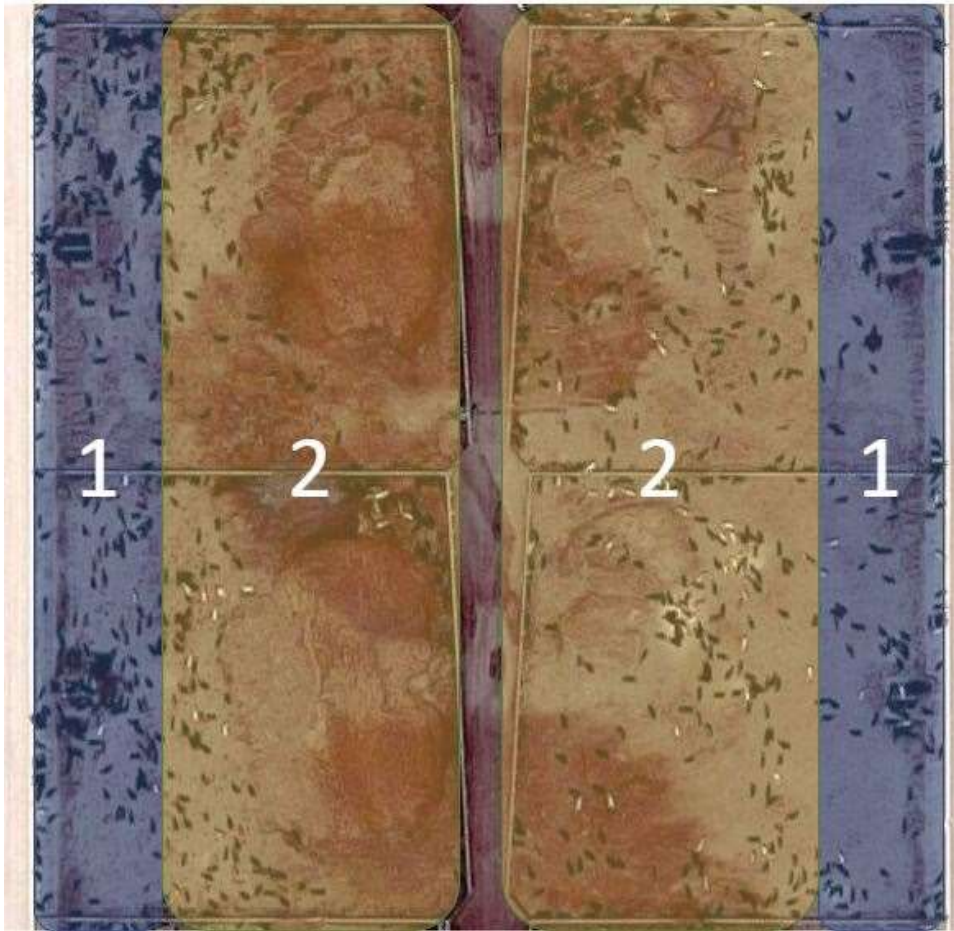
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APPENDIX

Appendix A: May Sample Collection- Zones



*Figure 25: May Sample Collection Zone 1 and 2*

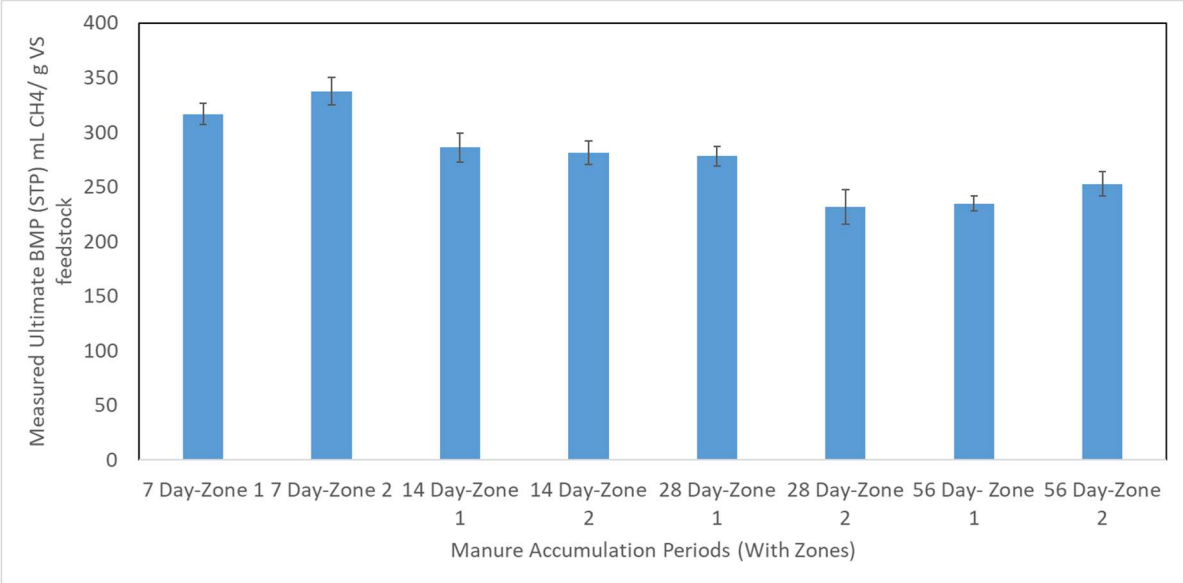


Figure 26: Ultimate BMP data for the May Sample Collection

Appendix B: 10 Year Average Temperature for Sample Collection Months

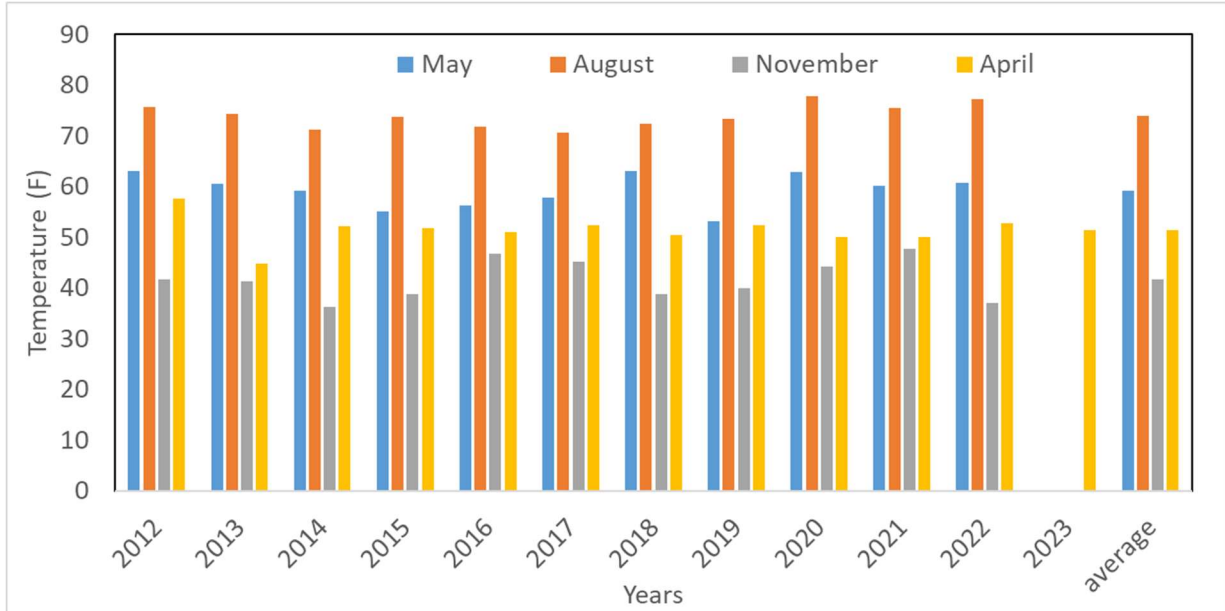


Figure 27: 10 Year Average Temperatures for Sample Collection Months

## Appendix C: Bottle Leak Test Protocol

### **Purpose of Protocol**

The purpose of this protocol is to test the Biochemical Methane Potential (BMP) bottles from leaking any gas out. The importance of BMP bottles is to contain the gas that is produced by the cow manure and transfer properly into the gas holding bags. If leakage occurs, it could skew the data collected.

### **Personal Protective Equipment**

#### ***Required PPE***

- Eye protection
- Long Pants
- Closed toe shoes

#### ***Recommended PPE***

- Gloves
- Lab Coat

### ***Protocol***

1. Locate the BMP bottles of interested.
2. Move the finalized BMP bottles to room 284, The finalized bottles should look like photo 1.
3. Find the gas gauge of interest which is SP-1 and take the long red tubing that is attached to SP-1 and attach it to the BMP cart.
4. Find the gas cylinder of interest, for the BMP bottles nitrogen will be used. The nitrogen cylinder is located in room 278A.
5. Above the nitrogen cylinder in room 278A, it states with gas gauge it is connected to in room 280, check that you are using the right gas gauge.
6. Next, turn on the nitrogen cylinder valve and watch the pressure increase. The pressure gauge can be seen in photo 3.
7. After that, go back to room 280 and turn on SP-1. Open all valves connected to the tubing and let the nitrogen go through them for a minute.
8. Once the tubing has been flushed, turn off the valve connected to the bottle. Then connect one of the BMP bottles by using the top valve seen in photo 1 and connect it to the tubing.
9. Open all the valve that connects from the BMP cart to the bottle to allow the nitrogen to come in and for the air to escape.

10. Attach the tubing to the BMP bottle and open the valve on the BMP cart, seen in photo 3. Turn it to .5 L/min for 3 minutes and then turn off all valves on the BMP bottle. Then turn off the valve on the BMP cart seen in photo 4.
11. Detach the bottle from the tubing and make sure all valves on the bottle are closed.
12. Take the BMP bottle and place it into a container of water upside down. The purpose of this step is to see if any portion of the lid is leaking (Photo 5). It is okay for some gas to leak from the tubing that is connected to the red valve. Any other leakage is a concern, and such needs to be fixed and retested.

## Appendix D: Bag to Bottle Leak Test Protocol

### **Purpose of Protocol**

The purpose of this protocol is to test the Biochemical Methane Potential (BMP) bottles to bag connection to determine the leakage rate. The importance of BMP bottles to bag is to contain the gas that is produced by the cow manure during the duration of the BMP run. There will always be a little leakage occurring due to an experiment setup however, it is important to have a minimum amount of leakage if possible.

## **Personal Protective Equipment**

### ***Required PPE***

- Eye protection
- Long Pants
- Closed toe shoes

### ***Recommended PPE***

- Gloves
- Lab Coat

## ***Protocol***

13. Locate the prepared BMP bottles connected to gas bags with tubing and proper fittings (BMP setup).
14. Place the BMP setup on a clean surface and where nothing will interfere with the setup for 3 days.
15. Check to make sure the red lid on the bottle is tightly placed onto the bottle and all connectors are tightly screwed on.
16. Label each BMP setup with a piece of tape either on the BMP bottle or gas bag.
17. Open up the bag valve 180 degrees of a full turn and the three-way valve to allow gas to go from the bottle to the gas.
18. Each BMP bottle has a tubing inside the bottle that is connected to the red valve. During the BMP run there will be a significant amount of liquid inside the bottle that will penetrate gas from going up the inside bottle tubing and out of the red valve connector. To replicate that process 100 mL of water was added to each bottle. The water is inserted with a 100 mL syringe with tubing on the tip to connect with the red valve. The red valve is then opened, and water will enter the bottle and push 100 mL of air into the gas bag.
19. Then use a 500 mL syringe to collect 500 mL of natural gas. The 500 mL syringe has a three-way valve connector, connected to the luer lock top of the syringe. Once the natural gas is obtained the three-way valve will be closed from all valves to make sure no natural gas and air mix.

- a. Natural gas is used instead of air due to the smaller particle size and more comparable to methane and carbon dioxide that will be generated in the BMP run.
20. Then take the 500 mL syringe and attach the three-way valve to the tubing on the BMP set up the three-way valve. Open the BMP setup valve towards the gas bag and then open the syringe valve so that the gas goes out of the valve connected to the BMP setup.
21. Once all gas has entered the bag the 500 mL syringe should still be attached. Use the syringe to pull out all the gas to make sure it is exactly 500 mL. If more than 500 mL of gas is in the bag let some of it go into the room and keep checking until it reads 500 mL.
22. After that close the bag valve and then disconnect the syringe. Place a weight on the bag such as a cardboard box to keep pressure on the bag.
23. Repeat process for how many BMP setups you want to test. It is important to note that all BMP setups will not pass this test so prepare extra bottles.
24. Record the time you placed the gas into the BMP setup and the temperature and pressure of the room. You will normalize the gas volume to moles to consider temperature and pressure differences that can occur.
25. Using the 500 mL syringe determine the amount of gas in each BMP setup at least once a day and record the same parameters. The test should run for at least 3 days which is the maximum amount of time gas would be in the BMP setup during the BMP run during GC testing.
26. For each day gas is sample it should be normalized to STP.
27. Then determine the leakage rate per day. If it is less than .5% per day, it was expected to be used during testing. If not, the BMP setup was fully examined for the next bag to bottle leak test.

Appendix E: GC Manual

### **Project Description and Scope**

The GC Machine is located in Room 284 on the second floor of the Suzanne and Walter Scott Jr. Bioengineering Building on CSU's main campus. The GC Machine is used for separation of gases to identify certain gases in a Sample. Dr. Sharvelle's Research Group will primarily use the GC Machine for Biomethane Potential (BMP) testing and analysis. Methane is the gas of interest for these projects.

### **Potential Hazards**

- Gas exposure
- Sharp Objects (syringe tips)
- Pressurized gas lines and gas cylinders
- Working with heavy gas cylinders

### **Personal Protective Equipment**

#### ***Required PPE***

- Eye protection
- Long Pants
- Closed toe shoes

#### ***Recommended PPE***

- Gloves
- Lab Coat

#### ***Start Up Procedure***

28. Locate the helium and hydrogen gas cylinders. They are located to the right of the GC machine and are the tallest two cylinders in the back of the cylinder grouping.
29. Turn on the helium and hydrogen gas cylinders by turning the valve handle located at the top of the cylinders. The pressure gauge will move, indicating gas flow into the GC machine.
30. Locate the air nozzle. The air nozzle is located to the left of the computer monitor on the rear of the bench near the electrical outlets.
31. Turn the air nozzle handle halfway so that it is aligned with the nozzle. The connected pressure gauge will move indicating air is flowing to the GC machine.
32. Once the gases and air are properly flowing to the GC machine, turn on the GC machine by pressing the ON button located at the bottom left of the front side. The button is not labeled ON but is rectangular and white in color. The upper right display will light up when turned on.
33. Once the machine is running, turn on the computer.
34. Once the computer is on, open the 6890 GC software (online).
35. The software will display a pop up when started, exit out of the pop up.

36. Once the software interface is showing, double click the method to be used for the analysis. The methods are listed on the left-hand side of the interface. The machine will make noise indicating it is loading the method.
  - a. For Dr. Sharvelle's research group, the primary method is JR\_Methane.M
    - i. The program will ask "Method DEF\_GC.M has changed. Save current changes?" select NO.
  - b. Notice the status bar in the top left corner of the interface. When the machine has properly loaded the method the status bar will read "Ready" and turn green in color. The loading process takes roughly 5 minutes.
37. When the software has loaded successfully you can begin analysis.

### ***Analysis Procedure (For testing standards)***

1. Locate the methane cylinder in the same grouping as the helium and hydrogen. The methane cylinder is the smaller of the three cylinders and is located at the front of the group. (Photo 2)
2. Check the red and white tubing valve to confirm that it is closed. A closed valve will have the red handle perpendicular to the valve body and tubing. An open valve will have the red handle parallel to the valve body and tubing. (Photos 4 & 5)
3. Once the valve is confirmed closed, turn on the methane by turning the valve handle located at the top of the cylinder. Methane will be released into the tubing.
4. The tubing needs to be purged of air. Open the tubing valve for 1-2 seconds to purge the gas from the tubing. Do this 2-3 times to purge all air from the tubing.
5. Before collecting the methane standard, confirm that there is a rubber septum on the tip of the 60ml syringe.
6. Once air has been purged from the tubing, keep the tubing valve closed and connect the 60 ml plastic syringe to the end of the tubing. The connection utilizes luer lock fittings (Photo 6). Connect the tubing fitting to the syringe fitting by inserting into the side arm of the syringe fitting. Once inserted, screw the fittings together to secure the connection. Be sure the white handle on the syringe luer lock fitting is closed. A closed luer lock will have the white handle pointing upward parallel with the tip.
7. Fill the syringe with methane by opening the tubing valve. The regulator on the cylinder should already be set to slowly release methane but be cautious of the pressure in the syringe as it could forcefully eject the syringe plunger.
8. Once the syringe is filled, close the luer lock fitting on the syringe by turning the white handle towards the side arm. Remove the syringe by unscrewing the fittings.
9. Purge the collected gas from the 60ml syringe by releasing it through the luer lock fitting. To release through the luer lock fitting screw open white portion. Repeat steps 5-7 twice. On the third collection, keep the methane sample in the syringe.
10. To inject the methane into the GC machine, it must be collected in a microliter gas syringe. Gas syringes have a needle tip. It is recommended to use a 100ul gas syringe.

These can be found in the cabinet below the computer monitor or on the desk next to the monitor.

- a. Dr. Sharvelle's research group has a designated 100ul syringe. It is in a "Hamilton" box labeled for the group.
11. Insert the needle syringe through the septum and into the 60ml syringe. Collect the methane with the needle syringe and remove from the 60ml syringe and septum. Be sure to minimize hand contact with the body of the syringes. Body heat can give a false reading due to an overheated sample.
12. The needle syringe needs to be purged. Release the methane that was just collected and keep the syringe closed.
13. Repeat steps 10 and 11 twice.
14. On the third collection keep the methane sample in the syringe.
15. Inject the gas into the GC Machine more information can be seen in the Calibration Procedure portion. To do this, locate the front injection port on the top left side of the machine. It is a metallic unit with a small injector at the top. Insert the needle completely into the injection port and inject the methane.
16. Remove the needle and immediately press start on the button pad of the machine. The button pad is located on the front right side of the machine underneath the digital screen. (Photo 14)
17. When the analysis is started it will run for a preset time. The status bar will read "In Progress" while the machine analyzes the gas. When the machine is done, the status bar will change back to "Ready".
18. When the machine is finished analyzing the gas, it will display a report. Press "Print" to save the file.
  - a. For Dr. Sharvelle's research group, save files to the LOET22 folder.
19. This completes the analysis process. To run additional standards, you can use the methane still left in the 60ml syringe.
  - a. Repeat steps 8-18.

### ***Calibration Procedure***

1. Open the LOET22 folder-> Analyses-> Calibration folder, once open create a new folder named today's date.
2. Open the Chrome, open the favorite tabs labeled "100 Services Gas Entry Services" excel file. Copy the sheet with the most recent data and name it today's date.
3. Fill the 100ul gas syringe with 100ul of methane from the 60ml syringe.
4. Once the computer says "Ready" in the top left-hand side of the screen, the gas syringe can be inserted into the front injector. After injected immediately press start.
5. When the machine is finished analyzing the gas, it will display a report. Press "Print" to save the file.
6. From the report copy and past the area from the second line into the new excel file.

7. Repeat steps 3-6 by changing the volume collected to 80ul, 60ul, and 20ul.
8. After all the areas are collected for all four volumes two graphs within excel will be generated. Which represents the line of best fit and the  $R^2$  value.

### ***Shutdown Procedure***

1. Ensure all data has been properly saved to the computer.
2. Double click the GC\_OFF Method located in the methods list.
3. The machine will stop making noises and the status bar will read “NOT READY”
4. Turn off the GC machine by clicking the button in the bottom left corner of the GC machine.
5. Wait for “Ready” to appear on the status bar.
6. Once the status bar reads “Ready”, turn off the air, helium and hydrogen going to the machine.
7. Once the gases are turned off, close the software.
8. This completes the shutdown procedure.

### ***Common Questions and Answers***

Question: If the GC machine states, “Status- Not Ready. Waiting for Ready. Front inlet flow, Front def flow....”

Answer: check the Helium and Hydrogen gas tanks pressure gauges. If one of them looks low turn the gas tank valve again to allow more gas flow. That tends to fix this problem.

Question: If the GC machine states, “Status- Not Ready.... Front Inlet Pressure”

Answer: First is to turn off the machine by selecting the GC\_OFF option on the computer. Once off, turn off the air nozzle and the gas cylinders. Next, check for obvious leaks in the septum or septum head fitting. It is most likely the septum needs to be replaced. To replace the septum, open the draw under the GC machine to the left.

Within the draw look for a small metal wrench and the septa packet seen in Photo 18.

Take the wrench and remove the nut from the front injection. Once removed take out the red septa and replace it with a new one, tweezers can be helpful for this portion. After the new septa is on use the wrench to put the nut back on.