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IMPACT OF PHYSICOCHEMICAL CHARACTERISTICS AND DISTILLATION PARAMETERS ON THE BIOMETHANE POTENTIAL OF BOURBON STILLAGE

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IMPACT OF PHYSICOCHEMICAL CHARACTERISTICS AND DISTILLATION PARAMETERS ON THE BIOMETHANE POTENTIAL OF BOURBON STILLAGE

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
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at the University of Kentucky

By

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Lexington, Kentucky

2023

ABSTRACT OF THESIS

IMPACT OF PHYSICOCHEMICAL CHARACTERISTICS AND DISTILLATION PARAMETERS ON THE BIOMETHANE POTENTIAL OF BOURBON STILLAGE

Bourbon, or whiskey, production in Kentucky has been estimated to double within the next five years and an increase in the main by-product from bourbon distillation, stillage. Stillage is composed mostly of water along with the fermented grains after distillation. Stillage is expensive to dispose of and difficult to store due to the high biodegradability, posing a risk to the environment given the low pH and high chemical oxygen demand (COD).

Anaerobic digestion has been identified as a potential solution for stillage valorization, but little research has been performed. Stillage from different mash bills has varying physicochemical properties, total solids (TS), volatile solids (VS), pH, and minerals and macronutrients. Distilleries employ varying distillation parameters and coupled with the heterogeneous makeup of the stillage from mash bills, is thought to have an impact on the biomethane potential of stillage. With a minimum methane production of 291.17±3.45 NmL/g VS and a maximum methane production of 419.19 ± 2.61 NmL/g VS out of 10 stillage samples from four distilleries with a food to microbe ratio of 1 g VS/ g VS and an organic loading rate (OLR) of 10 g VS/L, mash bill and distillation parameters were determined to impact stillage biomethane potential.

KEYWORDS: Bourbon, stillage, anaerobic digestion, biomethane potential, distillation

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04/16/2023

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DEDICATION

To my family for always pushing me to be the best I can be, my dogs for often adding extra stress but also providing never-ending excitement, and to the Barzee Lab Cake Day for always providing something to look forward to each month.

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CHAPTER 1. INTRODUCTION

1.1 Bourbon Industry in Kentucky

Worldwide, Kentucky is known for the bourbon produced, ranging from small, independent to large, corporate distilleries. In recent years, bourbon has become increasingly popular, enabling distilleries to expand and increase production, with production expected to double within the next five years [1]. Many new craft distilleries are opening their doors, while well-established distilleries are opening new distilling locations and warehouses with hopes they remain caught up with the demand. In 2009, Kentucky only had 19 licensed distilleries and in 2021, that number grew to 95, located in 40 of 120 Kentucky counties [1]. Most distilleries are located in central and northern Kentucky, with some in the western portion, creating a triangle of distilleries across the state, mostly located near the Kentucky and Ohio Rivers.

The bourbon industry arguably has the largest impact on the economy, with a total impact of 22,540 jobs across the state, $1.23 billion in payroll, and an economic output of $8.94 billion [1]. The jobs within the industry range from marketing, research and development, supply chain, engineering, human resources, shipping, considering Kentucky is a three-tiered state when it comes to the shipping of alcoholic beverages, and beyond. As of 2020, there were 10.3 million barrels in storage, all of which are taxed annually, contributing to the large economic output [1]. At last record in 2020, 2.4 million barrels were produced, with the most common barrel size being 53 gallons, equating to approximately 127.2 million gallons of bourbon produced in 2020 [1].

Despite the favorable distilled product created, a large amount of by-product, known as stillage, is produced. Worldwide, this has become a problem for the industry due to the lack of sustainable and economical ways to handle the stillage. Most often, stillage is provided to farmers as use for livestock feed, but distilleries are taking financial losses by doing so, and the amount of stillage produced is far outpacing the demand by farmers. Many distilleries, especially smaller craft distilleries, are in urban areas with no quick access to farms to deliver stillage to, and transportation is one of the greatest costs associated with providing stillage to local farmers. The city of Louisville,

Kentucky is home to the most distilleries within the state. Louisville is an urban area, with little immediate access to farms for stillage disposal, leaving them with few options. This issue is leading to high disposal costs and potential environmental risks. In Kentucky alone, over one billion gallons of stillage is produced annually by the bourbon industry, and as the industry expands, there is a critical need to identify the best practices to transform stillage into value-added products.

Other options for stillage disposal exist as well, but each of them possesses their own issues. Often, the main issue is the amount of time and energy required to perform those processes. Due to the various physicochemical properties of stillage, it spoils rather quickly unless handled nearly immediately, with a common shelf life of approximately two days. Refrigeration allows for some longer storage, but this is not feasible given the large volume of stillage produced daily at distilleries. Many utilize dry houses to remove some of the moisture content to increase stability of the stillage and reduce transportation costs for transporting the stillage to local farms. One main downside to utilizing a dry house is the amount of energy required for heating. Essentially, many other products are available to produce from stillage, but they are not suitable for long term growth within the industry. The stillage comes from the bottom of the still as a product known as whole stillage, and through various other processes can be converted into thin stillage, wet cake, and syrup, which all have various uses (Figure 1.1).

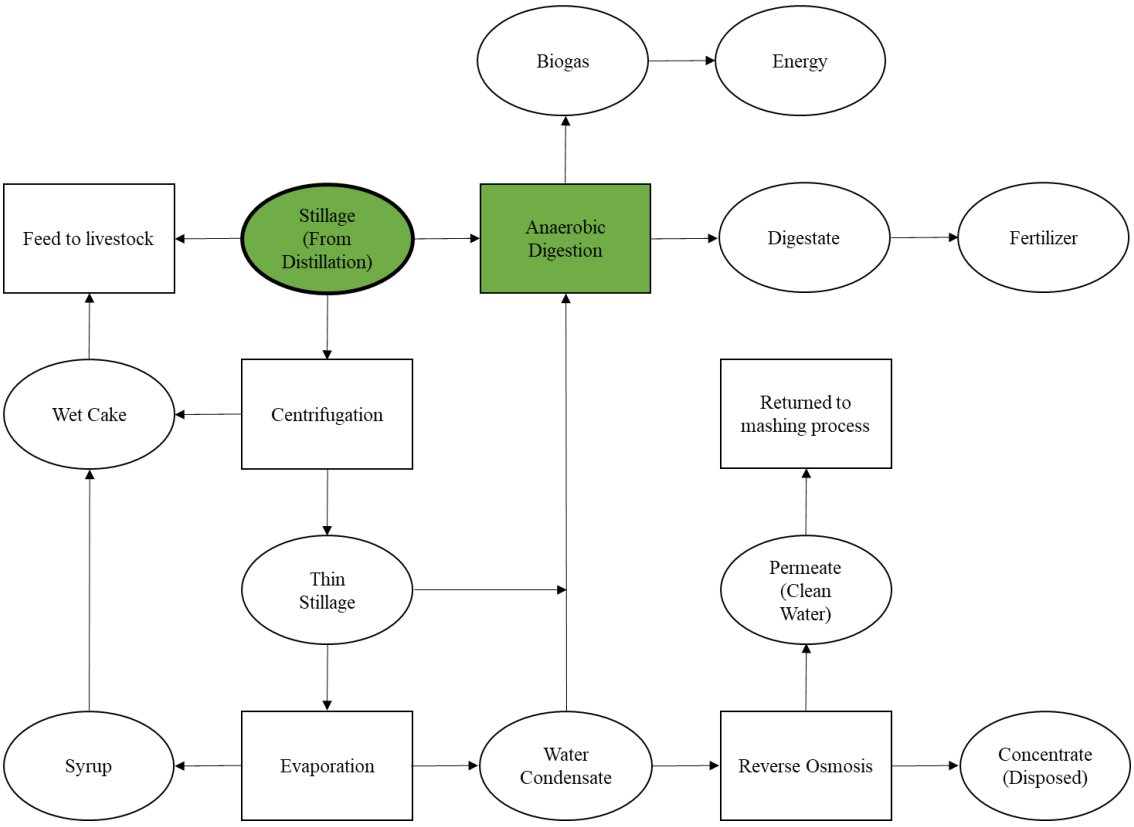

Figure 1.1 Flow diagram of common methods of stillage usage, including the focus of this thesis highlighted in green.

1.2 Project Overview

Due to the overwhelming amount of stillage produced, this project aimed at finding a solution to mitigate the bottleneck effect often caused by the overproduction of stillage. Anaerobic digestion (AD) was tested as a viable option to utilize the stillage outside of the conventional methods. The physicochemical characteristics of stillage such as moisture content, biodegradability, and nutrient content make it an ideal candidate for AD to produce renewable energy from biomethane as well as biofertilizer products. Overproduction of stillage has become the main topic of sustainability at distilleries worldwide, with all distilleries searching for a better way to use this waste.

Despite AD presenting itself as a possible solution, many questions remain such as which situations AD is best suited for, including if distilleries are centralized and located close together or not, and the production volume of the distillery. Considering each

distillery produces various bourbon or whiskey products with varying mash bills, stillage is a very heterogeneous product, and it is unknown if the varying mash bills will have a substantial effect on the physicochemical characteristics which commonly impact AD, or if various distillation parameters will be a key factor. Given these unknowns about stillage, this study aimed to address two objectives: (1) characterization and comparison of physicochemical properties of stillage collected from Kentucky distilleries and (2) determinization of the effect of stillage characteristics on its biochemical methane potential (BMP) with an analysis of the feasibility and viability of performing AD on stillage.

In performing the first objective, characterization, and comparison of the physicochemical characteristics of stillage, it will become clear how mash bills and operational parameters impact the physicochemical characteristics and in turn impact the BMP, the focus of the second objective. Upon determining biogas yield, a preliminary techno-economic analysis (TEA) can be completed to analyze the financial impact of implementing AD at a distillery, and the impact it could have on the surrounding communities. The goal is to assist the bourbon industry in finding a feasible alternative to create value from the overwhelming volume of stillage produced. Based on preliminary calculations and considerations, it is expected that subjecting stillage to AD will produce adequate amounts of methane to be utilized as bioenergy, and the various mash bills and distillation parameters will have an impact on the BMP.

CHAPTER 2. LITERATURE REVIEW

2.1 Bourbon Regulations and Creation

Contrary to popular belief, bourbon does not have to be made in Kentucky, although 95% of all bourbon is produced in Kentucky [1]. As designated by the Code of Federal Regulations, bourbon must consist of a mash (grain mixture) of at least 51% corn, distilled at 160° proof or less, and stored in brand new, charred white oak barrels at 125° proof or less for at least two years [2]. Formerly, it was thought that bourbon could only be made in Kentucky due to the limestone shelf, which impacts the flavor from the water, but due to updated technologies, some distilleries have rid this method, while others have maintained using water from the limestone shelf. Although most distilleries in Kentucky produce bourbon whiskey, some also produce rye, wheat, or malt whiskies, which require at least 51% of the respective grain (rye, wheat, or malt) and must be distilled at 160° proof or less, and aged in a charred, new oak barrel at 125° proof or less [2]. Sometimes, the whiskey created does not fall under any specific whiskey categories and can therefore be classified as just whiskey. The overall requirements for whiskey include being produced from a fermented mash of any grain distilled at less than 195° proof and stored in oak barrels, then bottled at a minimum of 80° proof (40% alcohol by volume) [2].

Most commonly, whiskey grains include corn, rye, wheat, and barley malt, but some organizations have begun including other grains such as rice or oats in mash bills. Distilleries across Kentucky use a wide variety of mash bills, as shown by 42 mash bills from 18 different distilleries (Figure 2.1). For the mash bills shown, corn appeared in 38 of the 42, rye in 33 of the 42, wheat in 10 of the 42, and barley appeared in all 42. Based on these mash bills, most of the distilleries in Kentucky produce bourbon or whiskey with corn, rye, and malted barley. Those that contain rye but no or little corn are not considered bourbon, but instead are considered rye whiskey if the mash bill contains at least 51% rye [2]. The mash bills accounted for were acquired from the websites of various distilleries in Kentucky, and only includes those that were explicitly mentioned on their general website.

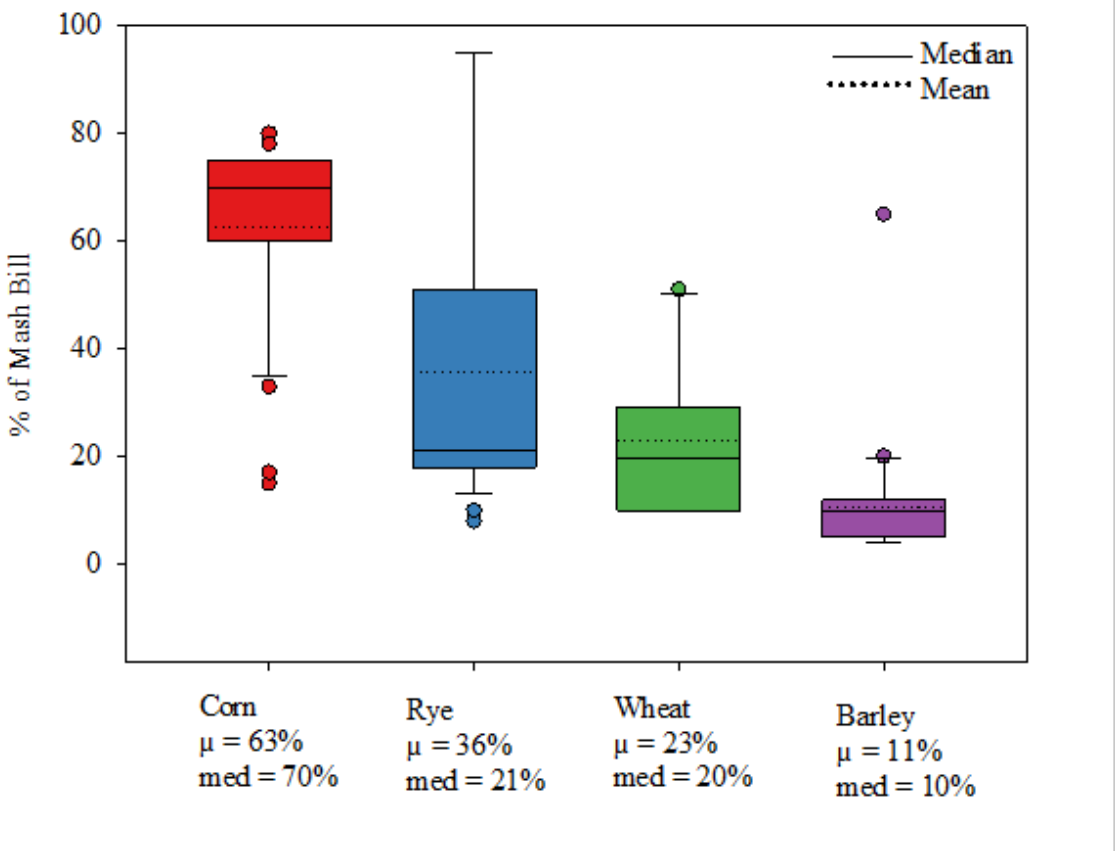

Figure 2.1 Box and whisker plot of the contents of varying mash bills across 42 mash bills from 18 distilleries in Kentucky.

Distilled spirits, including whiskey, are produced by a series of processes known as malting, mashing, fermentation, and distillation (Figure 2.2). Some distilled spirits transition into bottling after distillation, but whiskey must first be aged and blended before it can be bottled and sold. The malting process is often not done at the distillery, rather it is done elsewhere. Malting is essentially a controlled germination process, where barley is heated to allow germination to commence to produce enzymes necessary in the mashing step [3]. After malting, the malted barley is roasted for flavor production, and packaged to send to distilleries for use in whiskey production. For the enzymes in the malted barley to work efficiently, the sugars in the grains must be accessible. Upon receiving barley and grains for whiskey production, each distillery will often follow different variations of the same process.

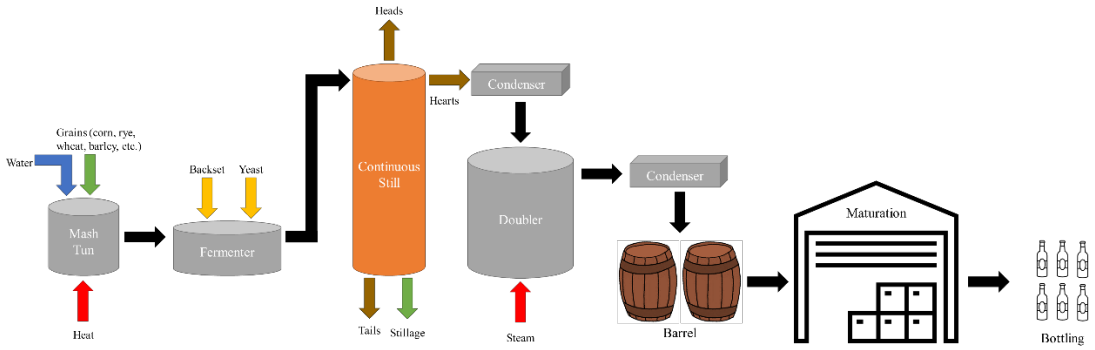

Figure 2.2 General overview of the distillation process from mashing to bottling.

These grains are milled to expose the surface area of starch in the grain, then heated in water in a mash tun with additional enzymes, which allows for the enzymatic hydrolysis of starch into simpler sugars such as glucose and maltose [3]. Mashing is crucial to whiskey production because yeast lacks the ability to hydrolyze starch molecules into monosaccharides and disaccharides. The process of gelatinization occurs next, where starch molecules are made readily available for yeast, and insufficient mashing results in starch granules not gelatinized, but overheating of the grain during mashing can result in the caramelization of sugars, reducing sugar availability to yeast [4]. Mashing serves two main functions: (1) releasing enzymes from malted barley and (2) utilizing enzymes from the malted barley for enzymatic hydrolysis of starch into simpler sugars for use in fermentation [4].

Upon completion of mashing, the mash (wort) is cooled for fermentation, to approximately 20 - 30°C, which is the ideal temperature range for the yeast, *Saccharomyces cerevisiae*, the common yeast strain used in ethanol fermentation [3]. Fermentation usually occurs for two to three days in large stainless-steel or cypress vats, where the yeast consumes the available sugars to convert to ethanol [3]. A common practice among American whiskey distilleries is the creation of a sour mash, which is the addition of backset which includes nutrients, organic acids and lowers the pH [3]. If not careful, other various bacteria can inhabit the mash, producing other off-flavors and in turn, ruining the end-product [4].

Once the yeast has consumed sugars for ethanol production, distillation begins, which can be completed in various ways. The whiskey industry uses two types of stills for distillation, continuous (column) stills or pot stills. Most often, distilleries use column stills, but few still use pot stills. Regardless of the equipment used, distillation is performed to separate the ethanol from water and other volatile compounds such as methanol. Distillation allows for the evaporation of other molecules that are dissolved in liquids to be separated [3]. For test or small batches, pot stills are used most often, consisting of a pot style base that narrows at the top, with a swan neck connecting to an arm, leading to a condenser [5]. Distilleries aiming to produce high volumes of a few whiskies more often use column stills, which allows for continuous distillation, not one single volume, allowing for increased product throughput [3]. Regardless of the still, both are typically made of copper, allowing for optimal distillation. Copper has high malleability and thermal conductivity, and has the ability to react with volatile compounds and sulfur produced, removing them from the distilled product [3, 5].

With the goal to separate ethanol from the compound, distillation must occur above the boiling point of ethanol, 78.4 °C, and is commonly performed at temperatures close to the boiling point of water (100 °C) [3]. Methanol, which has a lower boiling point than ethanol, is also present in the fermented mash and is harmful to consume, therefore it must be removed. To ensure any undesired products are removed while the desired product of ethanol is retained, the distillate is separated into the heads, hearts, and tails. The heads are the most volatile compounds with the lowest boiling points, including methanol, acetone, and acetaldehyde, and are removed from the still during distillation [3]. After the heads, the hearts run thought, containing mostly ethanol. Since this is the desired product, the hearts are sent to a condenser to be used in the final product [3]. Lastly, the tails contain the products with the highest boiling points, including some water, and is often redistilled to recover any remaining ethanol [3]. At the end, the grains and remaining water at the bottom of the column or pot still is removed.

After proper distillation and condensation, the condensed distillate is added to a barrel, specifically a new, charred, American white oak barrel for bourbon whiskey. Frequently, barrels are 53 gallons in size, but some craft distilleries will use smaller

barrels for the sake of cost reduction and development of different flavors, due to the change in surface area contact with the liquid [5]. This barrel is aged for at least two years then removed from the barrel and blended. Blending often includes the mixing of one or more barrels of different mash bills to produce various flavors, or the addition of water to ensure the whiskey is bottled at the proper alcohol concentration. While the bottles of whiskey hit the shelves at retailers, the excess of stillage remains to be handled.

2.2 Anaerobic Digestion Overview

Anaerobic digestion is process in which microorganisms such as bacteria and archaea biodegrade materials such as animal manures, biosolids from wastewater, and food wastes in conditions of no oxygen (anaerobic conditions) [6, 7]. This biological process is completed via four steps known as hydrolysis, acidogenesis, acetogenesis, and methanogenesis [8] (Figure 2.3). Through these processes, two main products, biogas and digestate are produced, with the biogas consisting mostly of methane (50% - 70%) and carbon dioxide (30% - 50%), with occasional trace amounts of other gas such as hydrogen (H_2) or hydrogen sulfide (H_2S) [9]. Both biogas and digestate produced have uses that improve sustainability of by-products from wastewater treatment plants, food production, animal agriculture, and other industries. Biogas can be used as an energy source, either by utilizing it as fuel for combined heat and electricity, or by upgrading and cleaning to gas to use as biomethane [9]. Since the raw biogas is not pure, various impurities such as water, hydrogen, and sulfide must be removed before use as energy. Once purified, biogas produced during AD makes for a great renewable fuel that is environmentally friendly [9].

Digestate, which consists of the remaining solids, contains high levels of nutrients, making it an ideal product for fertilizer [9]. Often, since the digestate contains the microorganisms needed for AD, the digestate can be recycled through the AD process as the inoculum, especially in batch experiments performed in laboratories.

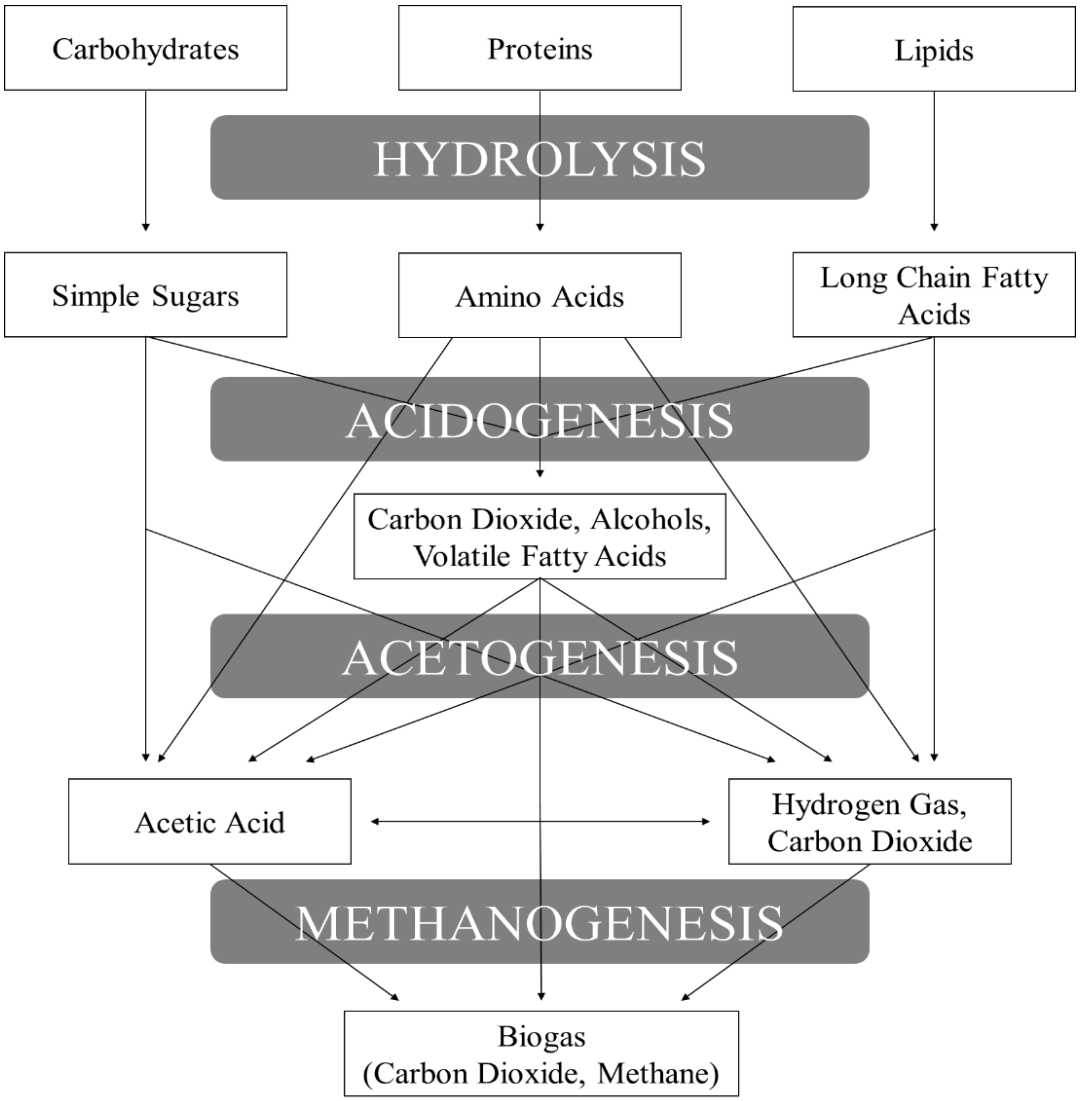

Figure 2.3 Flow chart of the four steps of the anaerobic digestion process, hydrolysis, acidogenesis, acetogenesis, and methanogenesis, along with the intermediate products created at each step.

During hydrolysis, large molecules are broken down into smaller molecules, and can be completed through the addition of water or through use of enzymes. In AD, this includes the breaking down of carbohydrates, such as starch and cellulose, proteins, and lipids into their smaller constituents of simple sugars, amino acids, and long chain fatty acids (LCFAs), a process that must be done for the microorganisms present to use the polymers for biogas production [10]. Typically, this is done in AD using enzymes produced by the bacteria present, including cellulases, amylases, proteases, and lipases

[8, 11]. Depending the molecules present, hydrolysis can be the rate limiting phase, increasing a lag phase in the beginning of AD [11]. Occasionally, the products of hydrolysis can be utilized by the bacteria and archaea directly to produce biogas, but most often they require to be further broken down or altered [11].

Once molecules are hydrolyzed into smaller constituents, they are converted into higher organic acids such as propionic and butyric acids through a process known as acidogenesis. This process is carried out by acid producing bacteria [11]. The over-production of higher organic acids, or volatile fatty acids (VFAs) is a common cause of failure for many digesters due to the drop in pH caused by the accumulation, making methanogens unstable and unable to produce methane [8]. Common metabolic pathways for acidogenesis include the conversion of glucose to ethanol and glucose to propionic acid [9]. Simultaneously, acetogenesis occurs, producing H_2 and acetic acid from the simple monomers produced during hydrolysis and the VFAs produced in acidogenesis, making them more available to methanogens for methane production [8, 9]. Acetogenic bacteria, the microorganisms that perform acetogenesis, can be inhibited by the H_2 gas produced, but many methanogens use the it for methane production [11]. If methanogens are operating optimally, there is a low risk of H_2 accumulation, with a low risk of acetogen being inhibited. The production of methane from H_2 is greatly dependent on pH, considering a low pH inhibits methanogens, further inhibiting the conversion of H_2 to methane [10]. When the ratio of propionic acid to acetic acid remains below 1.4, pH remains stable, and VFA inhibition does not occur [9]. When acidogenesis and acetogenesis occur simultaneously and efficiently, methane production is not hindered by the accumulation of VFAs.

In the final step, methanogenesis, methanogens convert the products of acidogenesis and acetogenesis into methane and carbon dioxide [8-10]. If any of the previous steps fail or are hindered, methanogenesis does not occur at an optimal rate. Methanogens are obligate anaerobes, where minimal exposure to oxygen renders them unable to metabolize acetic acid for methane production [8]. Not only are methanogens sensitive to oxygen, but also to pH, the main contributing factor to a desired reactor pH of 6.8 – 7.2, with tolerances as low as 6.5 and as high as 8.0 [9]. Once biogas accumulation

begins to slow due to decreased substrate availability, the reactor is said to have reached the biomethane potential which varies in time based on the conditions the reactor is held in.

2.3 Optimization of Anaerobic Digestion

As with most biological processes, various parameters are considered for optimal production. First and foremost, AD requires anaerobic conditions, considering the microorganisms present are mostly strict and obligate anaerobes [11]. There are many design considerations when deciding to implement AD, including the characteristics of the material, loading variations, organic concentration, temperature, alkalinity, availability of nutrients, and the expected and desired methane gas production [12]. With the microorganisms present in AD sensitive to oxygen, pH, and temperature, it is crucial to understand which parameters optimize AD.

Often, changes to the FM ratio, OLR, temperature, and pH can improve the process, as well as the addition of additional nutrients or substances. For temperature, AD can operate under mesophilic or thermophilic temperatures, and each have their benefits and drawbacks. With mesophilic temperatures, AD often progresses at a much slower rate, but is less energy intensive due to the lack of energy needed to produce higher temperatures. Mesophilic AD occurs at temperatures closer to room temperature, and has been found effective, especially with the addition of iron and chlorine [13]. Under thermophilic conditions, AD often progresses much quicker, but requires more energy for heating to higher temperatures. Overall, it has been discovered that thermophilic AD causes a greater reduction in the chemical oxygen demand (COD) than mesophilic AD, especially when cobalt was added to the system, increasing the overall efficiency of the BMP [14]. As noted, both temperature and mineral or nutrient addition can greatly impact AD efficiency.

When adding nutrients to the reactor, microorganisms in the system are provided with additional food sources to better thrive and produce the desired products. The addition of some nutrients in high or low amounts can allow for better production, depending on the nutrient, with AD most effective with magnesium, potassium, and

cobalt added in higher concentrations, iron and nickel in low concentrations, with no addition of sodium, manganese and copper, when digested with brewers spent grains (BSGs), a by-product of the brewing industry [15]. Many substrates naturally contain minerals such as calcium, magnesium, potassium, sodium, copper. While some minerals are beneficial, others present themselves as antimicrobials, in turn inhibiting AD. Two common minerals, copper and zinc, were both found to inhibit the co-digestion of waste activated sludge and septic tank sludge when present in levels of 20 to 80 ppm [16]. The ratio of Carbon and Nitrogen (C/N) is equally important in AD. Too high of a C/N could mean high levels of carbohydrates which can be difficult to degrade, while too low of a C/N could indicate high levels of nitrogen, which often leads to nitrogen inhibition; keeping the C/N between 25 and 30 often aids in reducing these problems [9].

2.3.1 Common Pretreatment Methods

Although AD alone can be an efficient process, pretreatment methods can be done to further increase yield or increase the rate at which the yield is achieved. Pretreatment methods include mechanical, chemical, enzymatic, and thermal options. In one study, BSGs were thermally pretreated by heating the substrate before performing AD, and increased the methane production by heating the BSGs up to 140 °C [17]. At pretreatment temperatures lower and higher than 140 °C, the methane yield decreased, with optimal yields at 140 °C [17]. While BSGs have a higher variety of grains than stillage or DSGs, the composition is similar in the sense that they are both composed of various grains and water.

Within spent grains, some yeast cells remain, which are difficult to break down for digestion. In enzymatic pretreatment of spent grain with protease and beta-glucanase under the conditions of a pH of 7 and temperature of 37°C, 90% of the yeast cells were lysed, which in turn reduces the COD [18]. A reduction in the COD increases biodegradability, allowing for easier anaerobic digestion and higher methane yields [18]. Chemical pretreatment of substrates is a common method to improve AD, as shown by Gunes in two forms of work using distillery byproducts, with the use of alkaline pretreatments [19]. The alkaline pretreatment was coupled with mechanical pretreatments such as mixing or thermal pretreatment such as microwaving [19, 20]. Alkaline

pretreatment in combination with mixing in the batch reactor, the amount of lignin present greatly decreased, allowing for greater production of biogas up to three times the amount of the control, due to the breakdown of the lignin by the alkaline pretreatment [19]. One form of alkaline pretreatment, the addition of sodium hydroxide (NaOH), in conjunction with microwaving, lignin removal and degradation was optimized, in turn optimizing the BMP [20]. Unfortunately, not all pretreatment methods are effective in improving the BMP, but they rarely hinder production. With the use of steam and sulfuric acid (H_2SO_4) as pretreatment methods, biogas production did not increase, but the rate of biogas production did, leading to a decreased retention time under mesophilic conditions [21]. In all, pretreatment methods typically provide the benefit of increasing biogas production by further degrading products that are difficult to degrade, or by increasing the rate at which biogas is produced.

2.3.2 Co-Digestion of Various Products

When acting alone, some substrates are not ideal candidates for AD, but when coupled with other substrates can produce large volumes of biogas. The mixing of two substrates for digestion is known as co-digestion, and often improves biogas yields when one substrate fails to do so alone. Co-digestion combinations include swine manure with corn stover, food waste with DSGs, cattle manure with food wastes, sewer sludge with cheese whey and BSG, among other numerous combinations for co-digestion. With the co-digestion of corn stover and swine manure at a ratio of 20:80, the highest yield of methane was obtained, but the mixture of 40:60 produced the most biogas total, proving that co-digestion of corn stover and swine manure is an effective means to achieving the BMP [22]. Other co-digestion options include the use of food by-products with DSGs; when combined at a ratio of 8:1 of DSGs to food by-product, the reactor was able to maintain a near ideal pH of 7.5, and produced a higher methane yield compared to other mixture ratios [23]. While co-digestion seems to be efficient most of the time, there are occasions where co-digestion reduces the methane yield. With adding leftover yeast to brewery wastewater as a co-digestion, the process does not seem positively or negatively impacted at a lab scale, but at a pilot scale, there was a noticeable decrease in the methane accumulation, due to the difficulty of hydrolyzing yeast cells [24]. For anaerobic

digestion systems that are not operating under ideal conditions, co-digestion could provide a solution.

2.4 Anaerobic Digestion of Distillery By-Products

Thus far, little work has been done pertaining to the AD of distillery by-products, specifically stillage. Despite the issue present worldwide, the minute amount of literature present focuses on whisky and scotch distilleries in the United Kingdom, where the distillery by-products are known as pot-ale, spent wash, and spent grain. The pot-ale is the material most closely related to whole stillage, while spent wash is mostly water, and spent grain is composed mostly of the grain remaining after distillation, where most of the moisture content has been removed from the stillage by filtering, centrifugation, or drying.

Notably, one distillery in Kentucky, Maker's Mark (owned by Beam Suntory), did support some research relating to AD of stillage, but they preliminarily deemed this unsuccessful. They cited common issues such as an accumulation of volatile fatty acids (VFAs) as a reason for failure, and did find methods to mitigate the accumulation, but at the time doing so still required the use of unnecessary energy in using a screw press to remove some moisture to utilize a thick slop instead of whole stillage [25]. In using a screw press to remove moisture, then subjecting the remaining solids to AD, a tCOD removal rate of 88-90% was achieved, AD showed the potential of producing 15-20% of the energy used by the distillery, and the use of the screw-press reduced energy usage by approximately 30% in comparison to drying for moisture removal [25]. Until recently, little has been discussed about the use of AD for the Kentucky bourbon industry. In the fall of 2022, Beam Suntory announced the implementation of anaerobic digesters to produce biogas for use as renewable natural gas (RNG), in hopes to power the facility, set to be complete in 2024 [26]. The distillery hopes to reduce greenhouse gas emissions by 50%, increase distillation capacity by 50%, and power the distillery with 65% RNG [26].

Despite the minimal research performed on the AD of stillage from whiskey or bourbon, few studies with related research have obtained similar results. In analyzing the AD of thin stillage and whole stillage produced at an Ireland distillery, the TS (wet basis)

of the thin and thick stillage were 3.19 ± 0% and 8.84 ± 0.03% respectively, with VS (dry basis) of 93.30 ± 0.21% and 95.62 ± 0.11% respectively [21]. From a different Ireland distillery, the TS and VS on a wet basis of the thin stillage was 3.9% and 3.5% respectively, with a TS of 8.8% and VS 8.2% of the thick stillage [27]. When subjecting the latter stillage samples to AD, the thin stillage had a methane yield of 494.6 ± 41 L CH4/kg VS, while the thick stillage produced 502.6 ± 42.7 L CH4/kg VS [27]. While samples from the first Ireland distillery were subjected to pretreatment methods, the non-pretreated samples produced high yields as well. For a mixture of draff, thin stillage, and thick stillage of 1:7:6 by weight, a final TS of 7.54% and VS of 95.72% were produced, leading to a mesophilic methane yield of 389.1 ± 8.5 mL CH_4/g VS [21].

Two additional studies used equipment similar to that of the Bioprocess Control Gas Endeavour (BPC GE), the Bioprocess Control Automatic Methane Potential Test System II (BPC AMPTS II) [28, 29]. One study tested the impact of how individual phenolic acids inhibit the biomethanization of distillery stillage, by adding phenolic acids to the stillage before AD in varying concentrations, with the BMP operating over 21 days [28]. Although the study focused on the impact of phenolic acids, the data from the control samples of no phenolic acid added provided some insight into the BMP of thick stillage. The other study was testing the AD of distillery by-products, including thin and thick stillage, with a BMP of 41 days at 37°C [29]. Between the two studies, there was a difference of 80 mL/g VS, likely a result of the different BMP times.

While these studies analyze the BMP of different whiskey byproducts (Table 2.1), little is still known about the impact of the different mash bills and distillation parameters on the physicochemical characteristics, which then impact the BMP. Although many studies provided data on some of the physicochemical characteristics of the stillage used, the mash bill was not provided, and one stillage sample from the distillery was analyzed. The data from these studies provided a gateway into the AD of stillage of varying mash bills, and a guideline for the expected methane yields from thin and thick stillage.

Table 2.1 Results of BMPs relating to stillage as found in literature.

Sample Type	TS (% wb)	VS (% wb)	Methane Yield (mL/g VS)	Source
Thin Stillage	3.9	3.5	494.6 ± 41	[27]
Thick Stillage	8.8	8.2	502.6 ± 42.7	[27]
Thin/Thick/Draff Mix	7.54	95.72[a]	389.1 ± 8.5	[21]
Thick Stillage	7.99	7.62	423	[28]
Thin Stillage	3.54	3.20	495	[29]
Thick Stillage	7.87	7.41	503	[29]

a: provided on a dry basis

2.5 Energy

2.5.1 Value of Energy

Biogases, especially methane, when converted can be utilized as an energy source, often as a renewable natural gas (RNG). Typically, methane will yield 10 kWh of energy (electricity) per 1 m^3, depending on how it is processed, typically with an electricity conversion efficiency of 35% [30]. If we assume the 2021 average household energy consumption of 10,632 kWh annually, it would take 1,063.2 m^3 of methane to produce enough electricity for one household in the United States [31]. For natural gas production, it is assumed that CH_4 has a near 100% conversion efficiency with an LHV of 36 MJ/m^3 CH_4, which equates to approximately 2930 MMBtu/m^3 CH^4 [32]. Each state has varying energy costs, but the average cost of electricity at the residential level for the United States is 15.64 cents per kWh, or 9.12 cents per kWh for Kentucky [33]. At the industrial level, the cost of natural gas is $4.99/MMBtu, with the cost of electricity at 7.07 cents per kWh [33, 34].

2.5.2 Energy in Distilleries

In the distilling industry, electricity and natural gas are used to operate the distillery, ranging from lights to the distillation columns. Arguably the most energy intensive process is drying, which is performed on the stillage as a means to remove water and stabilize the stillage. One Kentucky distillery was estimated to use 176,400 kWh of electricity just by operating the centrifuge to remove most of the moisture from the stillage [35]. Another distillery used 10,000 MMBtu of natural gas per month, or 120,000

MMBtu annually, with a fluidized bed dryer as a means of drying [35]. These aspects just capture portions of the energy used for a distillery operation. Using the estimated cost of energy above, the first distillery would spend at least $12,000 annually on electricity, not including all electrical or natural gas requirements, while the second would spend nearly $600,000 on natural gas. Although these numbers may vary based on how much is produced annually at the distillery, the amount of energy used by a distillery becomes costly, and in using nonrenewable energy sources, becomes a threat to the environment.

CHAPTER 3. METHODOLOGY

3.1 Sampling & Characterization Methods

3.1.1 Sampling

The sampling process begins with communicating with the distillery of choice, setting up a date or time to retrieve the stillage sample. Unless the still has been shut down for some time, the stillage is removed from the still at approximately 200°F (93.3°C). Due to the potential risks of transporting material at that temperature, most distilleries will remove the stillage from the still prior to arrival, to allow for slight cooling of the material. The ideal time to retrieve the sample is the day of the most recent distillation, to ensure freshness of the stillage, considering the high degradability of the stillage over time. Drying and freezing of the stillage could alter the properties and was often used as a pretreatment for anaerobic digestion, which was not ideal for this study. The sample amount for each stillage was approximately three to four gallons. Often, due to distillery operations, stillage samples might have been a day or two old, but were often kept in conditions that minimized degradation, such as a holding tank, where the sample was taken at a temperature of approximately 150°F (Distillery D). Although some sampling aspects were under the control of the distillery, effort was made to ensure representative samples were taken that were similar across the distilleries.

3.1.2 Stillage Characterization

Upon sampling, tests were performed on the stillage to determine the parameters of the BMP. After retrieving the samples, they were cooled to approximately room temperature. As they cooled, a sample is taken for photography to analyze the physical properties, and pH was measured using the Thermo Scientific Orion 8102BNUWP Ross Ultra Combination pH probe [36]. Prior to using the pH probe, calibrations were performed as outlined in the manual, using the provided buffers of pH 4, 7, and 10. Once cooled, the five-gallon bucket of the stillage was placed in a walk-in refrigerator that maintains a temperature of 34°F. Effort was made to reduce temperature as close to room

temperature before placing in the refrigerator to prevent any potential damage to other samples in the area. The next day, stillage characterization began.

On day one, total solids (TS) were performed. The determination of TS was performed using method 2540 outlined in the American Public Health Association (APHA) Standard Methods for the Examination of Water and Wastewater [37]. Using a balance, 75 mL disposable aluminum weighing dishes were weighed (in grams). The stillage was thoroughly mixed, and a sample, approximately 10 mL, was taken with a pipette controller, and added to the weighing dish. Each stillage sample utilized duplicates for determining TS. They were then placed in the oven at 105°C for approximately 24 hours. Afterwards, the samples were placed in a desiccator to prevent any excess moisture uptake from the surroundings during the cooling process. The samples were weighed and recorded, allowing for the calculation of TS (Appendix II). At the same time, the inoculum underwent TS following the same method, but was completed in triplicates due to the nature of the consistency of the inoculum.

After the completion of TS, the test for determining volatile solids (VS) was completed on day two. Using the same dried samples from the TS determination, the weighing dishes were placed into a furnace at 550°C for three hours. After the three hours was up, the furnace cooled to 105°C, a temperature much safer for handling, which also took approximately three hours to do. Afterwards, the samples were placed in the desiccator until cooled, then weighed and recorded. Using the values from the TS calculations, VS could be calculated (Appendix II). Once VS had been calculated for the stillage and inoculum, the loading of the substrate and inoculum could be calculated to fit the desired parameters of the BMP (Appendix III).

While knowing VS is a crucial aspect of performing a BMP, considering that is the determining factor for the loading rate in this experiment, understanding the mineral composition is also important. Mineral composition is crucial for understanding possible inhibition during the BMP. Utilizing the animal feed division at the University of Kentucky Regulatory Services, mineral composition along with macro-nutrients (fat, fiber, and protein) was found, using one gallon of well-mixed stillage, sampled from the five-gallon bucket acquired from the distillery. The minerals considered were calcium

(Ca), phosphorus (P), sodium (Na), potassium (K), magnesium (Mg), copper (Cu), cobalt (Co), iron (Fe), manganese (Mn), and zinc (Zn). Using a microwave digestor and inductively coupled plasma – optical emission spectrometry (ICP-OES), Association of Official Analytical Chemists (AOAC) method 2017.02 was followed [38]. The other analyses, such as protein, crude fiber, acid detergent (AD) fiber, neutral detergent (ND) fiber, and fat were found using an in-house method for near-infrared spectroscopy (NIR). The results consisted of a table identifying the units of the findings, if the value was a guaranteed maximum or minimum, and the value found. This characterization was only performed on the stillage and not the inoculum. Results for the analyses from Regulatory Services typically took three weeks to complete, but the BMP could be started before having the results from the characterization. Carbon and Nitrogen tests were performed by the Department of Plant and Soil Sciences at the University of Kentucky utilizing a combustion based method outlined by the Soil Science Society of America [39].

3.1.3 Pre-BMP Characterization

On day three, the BMP samples were prepared and analyzed before beginning the experiment. From each unit, a sample of approximately 10 mL was taken to perform TS and VS to utilize for comparison at the end of the BMP, for determining the VS destruction. For understanding how the pH changes during the process, pH was measured for each unit, and to ensure the units are starting out at a neutral pH level. A pH that is too low can inhibit biogas production, therefore understanding the pH of the system is important. Due to limited biogas production during the first phase of the BMPs, all second phase BMPs underwent an alkalinity test using method 2320 of the APHA (1999) [37]. For alkalinity, 0.1 N of H_2SO_4 was used, and due to the time and resources required, composite samples were utilized. Since triplicates were used, 15 mL from each related sample was taken, for a total of 45 mL of sample for alkalinity. Only 25 mL of the 45 mL was used, then titrated using the 0.1N H_2SO_4 until a pH of at least 4.5 was achieved, usually lower to allow for an accurate linear interpretation and calculation of the alkalinity of the sample (Appendix IV).

3.1.4 Post-BMP Characterization

Upon completion of the BMP, following the same APHA method, TS and VS were calculated for each sample, and pH was measured again to determine if the net pH increased or decreased during the experiment. Alkalinity was not performed, but ammonia concentration was measured following the analytical techniques for the Thermo Fisher Scientific High Performance Ammonia Ion Selective Electrode, creating composite samples of 15 mL from each unit for a total of 45 mL [40]. Although the manual recommends using 100 mL of the sample for testing ammonia, this was scaled down to 40 mL of sample, while using 0.8 mL of the pH-adjusting ISA. To understand the qualities of the digestate, composite samples of 15 mL from each sample are taken, for a total of 45 mL, and sent to the soil lab of Regulatory Services at the University of Kentucky. The samples were subjected to liquid animal waste testing which includes nitrogen, phosphorus pentoxide (P_2O_5), potassium oxide (K_2O), calcium, magnesium, zinc, copper, manganese, and total carbon. Analysis was performed using the Recommended Methods of Manure Analysis (A3769) from the University of Wisconsin [41]. The results were provided in pounds per 1000 gallons (lbs/1000 gal) on a wet basis then converted to grams per liter (g/L) on a dry basis. The use for the digestate is out of the scope of this project but provides an idea of how the substance changes over time during the BMP. The remaining digestate not sent for characterization was then centrifuged at 4500 rpm for 15 minutes to concentrate for use as the inoculum in the following BMPs.

3.2 Anaerobic Digestion Methods

The BMPs for this experiment were carried out using the Bioprocess Controls Gas Endeavour (BPC GE) [42]. This unit consists of three major parts: the sample incubator, gas absorption unit, and the gas measurement device (Figure 3.1, Figure 3.2). The sample incubator is where the digestion occurred, with 15-500 mL bottles, each with a working volume of 400 mL, in a water bath maintained at 30°C. Each unit also had a motor with a stirring bar attached, set at 100 rpm, to alternate between clockwise and counterclockwise every 5 seconds for one minute every ten minutes. Tygon® tubing with an inner diameter

of 3.2 mm and outer diameter of 6.4 mm connected each to a 100 mL bottle, the gas absorption unit. These units are designed to absorb any undesired gases, such as carbon dioxide (CO_2) and hydrogen sulfide (H_2S). Since CO_2 is the most frequently produced gas along with methane (CH_4), a 3M solution of potassium hydroxide (KOH) was used, with the pH indicator Thymolphthalein, as recommended in the BPC GE manual [42]. Additional Tygon® tubing connected the gas absorption to the gas measurement devices, which are volumetric flow devices that measure the gas accumulation and gas flow.

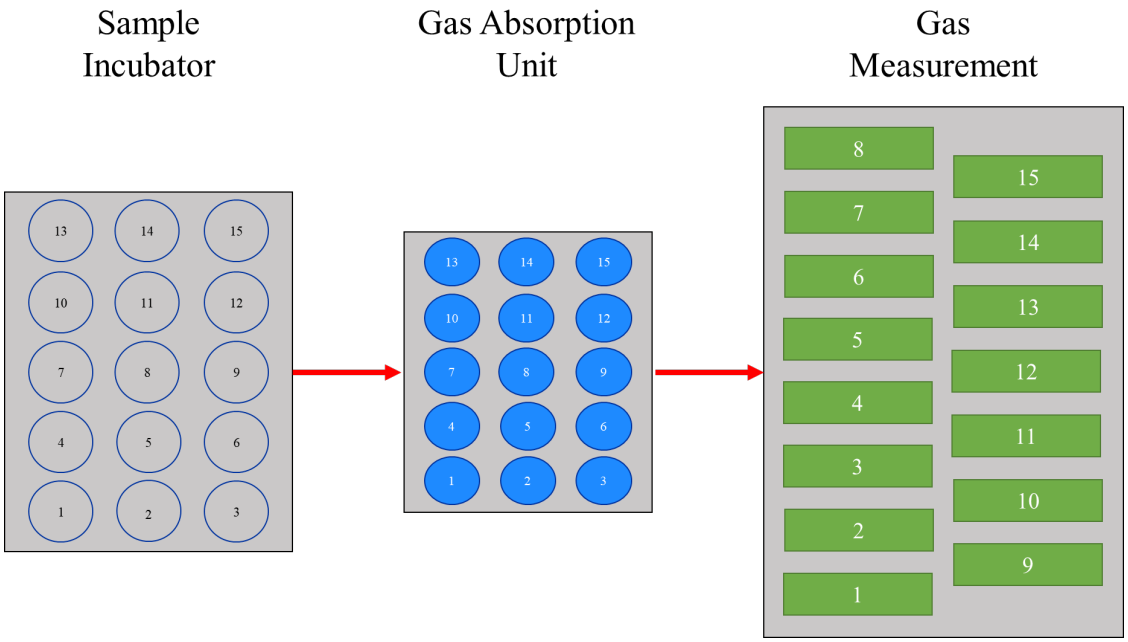

Figure 3.1 Flow diagram of the units of the Bioprocess Control Gas Endeavour system as it was set up for the BMPs.

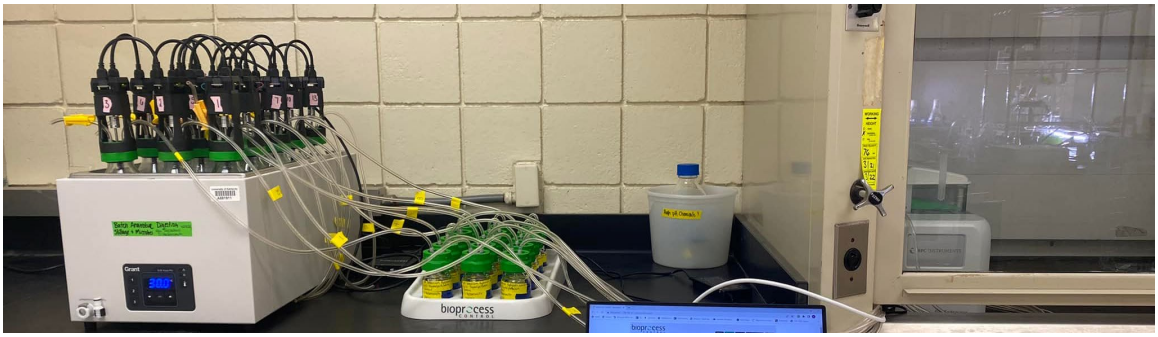

Figure 3.2 Setup of the Bioprocess Controls Gas Endeavour in the lab with the 15 sample incubators, gas absorption units, and the gas measurement device in the fume hood, which was connected to a computer for data access.

Each unit within the water bath was labeled one through fifteen, keeping a consistent numbering pattern across experiments. Each sample was randomly assigned a position in the sample incubator, positions one through fifteen. In the instance where there were less than fifteen samples, those locations were listed as “empty”, with the random number generator also randomly assigning the location of the empty units. This was done for various reasons, being the water bath could produce more heat in one area than another, and to account for a potential voltage drop across the motors since they were connected in series. The initial power source to the motors was connected to unit 15, then woven through to each additional unit (Figure 3.3). Therefore, it is possible that unit 15 had a slightly higher voltage than unit one, impacting the true speed of the stirring bar. By using a random number generator to spread out each sample, one individual group of samples does not receive higher speed stirring than others.

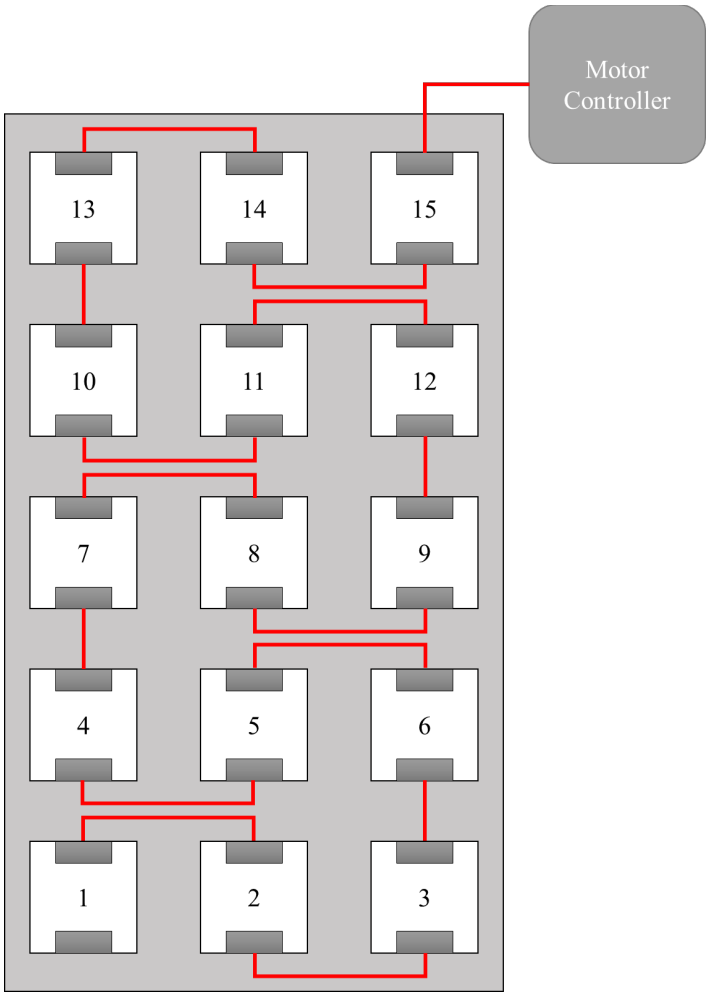

Figure 3.3 Wiring diagram of motors connected in series for the sample incubator units.

Upon completion of TS and VS, the desired FM and OLR on a VS basis were used for determining the amount of substrate, inoculum, and water needed for the working volume of 400 mL (Appendix III). Each sample was utilized in triplicates, typically with three different stillage samples, a positive control of cellulose, and a negative control. For each, the calculated amount of inoculum, water, and stillage (or cellulose for the positive control). Since the inoculum and stillage had a high moisture content, it was assumed the density of both were similar to water, therefore they were measured by volume (mL) instead of by weight (g). Once mixed, the preliminary testing of TS, VS, pH, and alkalinity was performed, then the motors with stirring bars were attached, along with the Tygon® tubing that connects to the gas absorption, and a shorter

piece of tubing with a clamp to serve as a sampling port if needed. The smaller piece of tubing was clamped to ensure anaerobic conditions were maintained.

Since oxygen was introduced to the system, once all parts were assembled, the motors were turned on for ten minutes to aid in flushing out any oxygen in the bottles or tubing. After ten minutes, the gas measurement was started on the online system the gas measurement device connected to. Each BMP was operated for four weeks (28 days), with the data checked periodically to ensure of no system failures. If gas production slowed or stopped, pH was checked to assess for possible VFA accumulations. If for any reason a unit had to be disassembled with the lid removed, the unit would be reassembled and sit in the water bath for ten minutes before resuming the gas measurement. The Tygon® tubing was also checked periodically for any back flow either from the gas measurement device or the gas absorption units and was flushed as needed. Each time a piece of tubing was detached, gas measurement would be paused for that individual unit and resumed ten minutes after reassembly.

On day 28, the end of the BMP, the gas measurement was stopped, and the final data file (.csv) was downloaded. Upon disconnecting all motors and tubing, pH of each was measured, along with TS and VS. A composite sample of 15 mL each was taken from each experimental unit to measure ammonia, and another composite sample of 15 mL each to send to Regulatory Services at the University of Kentucky for digestate analysis to later be used in future studies. Once all testing was completed, the remaining digestate was centrifuged at 4500 rpm for 15 minutes, with the supernatant discarded, to produce a more concentrated inoculum for the next BMP. In reusing the digestate as the inoculum for the next BMP, the microorganisms are already acclimated to the stillage and produce biogas more efficiently.

Since only up to three stillage samples can be tested at one time on the Gas Endeavour, seven BMPs were performed. Unintentionally, these BMPs were divided into phase one, parameter optimization, and phase two, with different stillage samples used at each phase (Table 3.1). Phase one BMPs were operated with the parameters of an OLR of 5 g VS/L and an FM of 1 g VS/g VS. Due to lack of acclimated inoculum in the early phase, the BMPs in phase one was a mesophilic inoculum from Quasar Energy Group,

produced from food by-products. After deciding those parameters were not allowing the samples to reach a methane potential, a separate BMP was performed to determine the optimal parameters for this study. The parameters considered were FMs of 0.25, 0.5, and 1 g VS/g VS and OLRs of 5 and 10 g VS/L, which were applied to each FM. This produced six experimental units, each with duplicates. The results from the optimization test showed an ideal FM of 0.5 g VS/g VS with an OLR of 10 g VS/L, based on output and feasibility. These parameters were chosen for the phase 2 BMPs. The data from each phase was compared individually, and results from the different phases were not compared between one another.

Table 3.1 Summary of the stillage samples used in each phase of the BMPs.

Phase 1	**Stillage(s) Used**
BMP 1	A1, B1
BMP 2	C1, C2
Parameter Optimization	
BMP 3	A1
Phase 2	
BMP 4	D1, E1
BMP 5	D2, E2, F1
BMP 6	F2, G1
BMP 7	D3, E3, F3

3.3 Data Analysis

During the BMP, the data was acquired through the Gas Endeavour system, accessed via ethernet to the gas measurement device. The data was downloaded as a .csv, showing daily data, including the gas accumulation (NmL) and daily gas flow (NmL/day). Through the system, the volume of gas was automatically normalized, accounting for pressure and temperature of the surroundings. Data for BMPs is best expressed per gram of VS (NmL/g VS); the data was altered to represent this by dividing the total amount of gas produced by the substrate loading rate. Since each experimental unit was triplicated, the averages and standard deviations were calculated. If the negative control produced any methane, that amount was removed from the total amount produced by the stillage samples. Often, the negative controls produced small amounts of methane due to the residual stillage in the inoculum from the previous BMPs.

For statistical methods, analysis of variance (ANOVA) was performed using the SAS analytical software. The ANOVA was performed at the significance level of 0.05, with a null hypothesis that the methane accumulation of stillage samples is equal, with an alternative. For further analysis into where the significant difference lies, post hoc Tukey tests were performed, also using the SAS statistical software. In determining the impact of various characteristics on methane accumulation, linear regressions were also performed.

3.4 Modeling

In order to model the kinetics of the BMP as it occurred, a modified Gompertz model was utilized:

$$y = Pe^{-e^{(\frac{R_m e}{P}(\lambda - t)+1)}}$$

Where y represents the expected methane yield (mL/g VS), P represents the maximum methane yield produced (mL/g VS), t represents the time (days), λ is the lag time (days), μ_m is the maximum growth rate (mL/g VS/day) and e is Euler's number (2.71828183) [43]. This equation was utilized in GraphPad Prism to fit the model to each stillage sample. The results shared were graphs of the actual and expected values, along with each parameter from the Gompertz model. The goodness of fit was determined with the R^2 and root mean squared error (RMSE).

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Physicochemical Characteristics & Distillation Parameters of Stillage

4.1.1 Stillage Characterization

In total, 14 stillage samples were acquired and tested, ranging from bourbons containing rye, wheat, brown rice, and rye whiskies. While most distilleries provided the mash bill, the makeup of the grains present in the stillage, Distillery D was unable to provide that information. Mash bills vary in grains and amounts, altering the classification of the whiskey produced (Table 4.1). Although others have the same classifications, the ratio of grains varies. Given the mash bills of each stillage sample, tests can be performed to determine how distillation parameters between distilleries impacts the BMP from samples E1 and F1, whereas all others can be used to test the impact of mash bill on the BMP within distilleries, since production should be similar within the distillery.

Table 4.1 Mash bills for each stillage sample, where Distillery D did not provide mash bill information, only the classification of bourbon produced. All corn is assumed to be yellow corn and barley is malted barley unless stated otherwise.

Stillage	Corn	Rye	Wheat	Brown Rice	Barley	Classification
A1	0	95	0	0	5	Rye Whiskey
B1	72	13	0	0	15	Rye Bourbon
C1	64	0	24	0	12	Wheat Bourbon
C2	39	51	0	0	10	Rye Whiskey
D1			Unknown			Rye Bourbon
D2			Unknown			Wheat Bourbon
D3			Unknown			Rye Bourbon
E1	67	23	0	0	10	Rye Bourbon
E2	55	0	0	36	9	Brown Rice Bourbon
E3	40	51	0	0	9	Rye Whiskey
F1	72	18	0	0	10	Rye Bourbon
F2	80[a]	10[b]	0	0	10	Rye Bourbon or Corn Whiskey
F3	17	65	0	0	18	Rye Whiskey
G1	75	0	21	0	4	Wheat Bourbon

a: red corn
b: malted rye

Although the mash bills of samples from distillery D are unknown, assumptions can be made to estimate the possible mash bill. Despite the names of “high” and “low” rye mash bills, the distillery did provide both mash bills contain less than 10% rye, meaning they likely have very high corn concentrations. Since very rarely does the malted barley concentration range above 20%, it can be assumed that the malted barley is less than 20%, and the corn content is less than 80% since it is not classified as a corn whiskey. Knowing this information, the mash bills for samples D1 and D3 can be estimated (Table 4.2).

Table 4.2 Estimations of the mash bills for samples D1 ("high" rye) and D3 ("low" rye) based on minimal information provided by the distillery and observations of other mash bills.

Stillage	Corn	Rye	Wheat	Brown Rice	Barley
D1	75	9	0	0	16
D3	79	5	0	0	16

When physical characteristics of D1, D3, E1, and F1 are compared, we can see that D1, the high rye bourbon, most closely resembles E1 whereas D3 most closely resembles F1 (Figure 4.1). Likewise, when the two presented wheat bourbons are compared, D2 has a much brighter yellow coloration than G1, leading to the assumption that D2 has a lower wheat content than G1 (Figure 4.2). Since corn has the highest percentage in bourbons, it can be assumed that the brighter yellow coloration in sample D2 means it likely has a higher corn content, thus lower wheat content. Several other factors can contribute to the coloration of the stillage, such as material of the still and time in the still, but grain concentration serves as an indicator of the color as well. It was also noticed that some stillages, such as those from Distillery F, have larger particle sizes which they credit to the use of a roller mill instead of the traditional hammer mill.

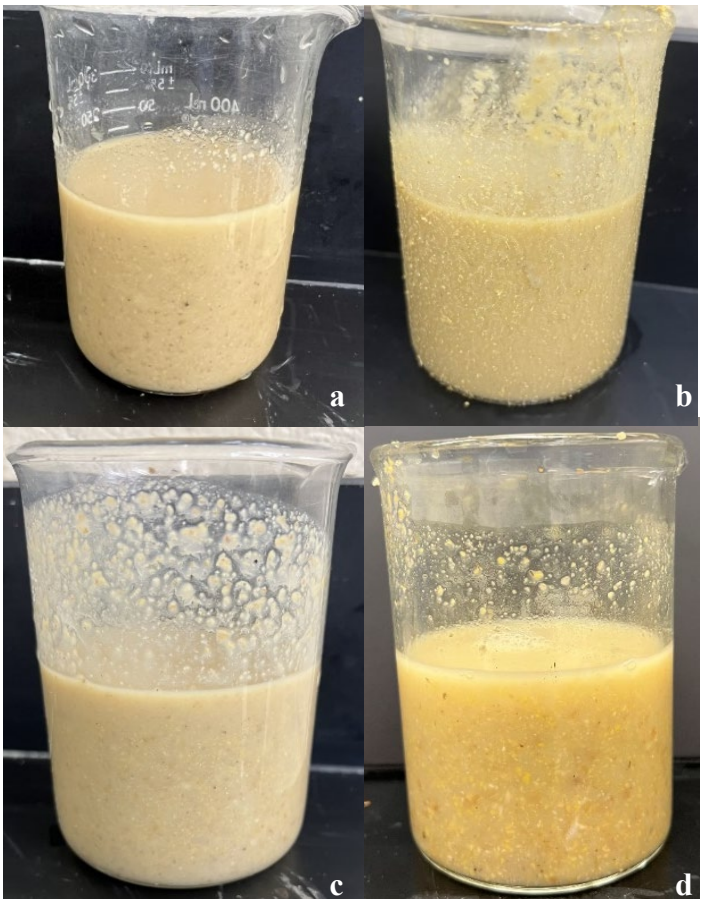

Figure 4.1 Rye bourbons D1 (a), D3 (b), E1 (c), and F1 (d) shown to be similar in color, and more of a tan color than wheat varieties.

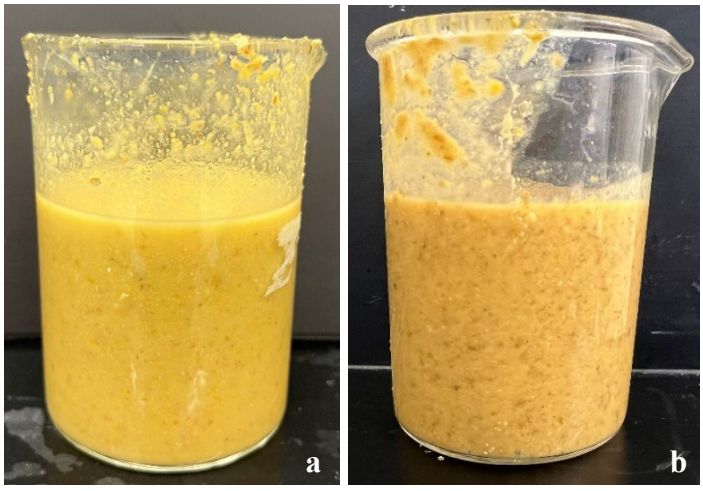

Figure 4.2 Stillage samples D2 (a) and G1 (b), both wheat-based bourbons, are shown to have more of a yellow appearance than rye varieties.

Thought to also impact stillage characteristics is the differing distillation parameters from each distillery. Since distillation relies on boiling off the ethanol to separate it, the temperature at the bottom of the column has to be around the boiling point of ethanol, but less than the boiling point of water, to minimize the amount of water retained. The temperature at the top of the column is most frequently measured as the vapor temperature, which is the temperature of the vapor before it enters the condenser. Each distillery provided information about their distillation parameters, with ranges provided for Distillery D (Table 4.3). Distillery G uses a pot still, making the parameters slightly different. For the top temperature, this is the temperature in the swan-neck of the still where the ethanol begins to condense. The height is the height of the actual pot still (4.5 ft) plus the height of the helmet (4 ft), with a much larger diameter than the column stills. Since the pot stills are not continuous, and all material is added at once, the flow was not relevant. Instead, they provided the information that it takes 5 to 6 hours to distill a batch that is 175 to 200 gallons. All others, C through F, use continuous (column) stills, with similar parameters to one another. Distillery C utilizes two columns with information for both columns provided, where C2 was produced on the smaller column, and C1 produced on the large column.

Table 4.3 Distillation parameters for distilleries C, D, F, and G. Information was not provided for distilleries A, B and E.

Distillery	Still Type	Top Temp (°F)	Bottom Temp (°F)	Height (ft)	Diameter	Flow (gpm)
C	Column	198	212	40	36 in 18 in	42.5 9.5
D	Column	190-195	195-205	N/A	N/A	330-360
F	Column	189	207	N/A	32 in	34
G	Pot	176	225-230	8.5	4.5 – 5 ft	N/A

The in-lab analysis of stillage, such as TS, VS, and pH were utilized to better understand the properties of the stillage for loading the BMP (Table 4.4). Each stillage sample varied in total and volatile solids, even within the distillery. The variability in the VS could impact the loading of digesters at the distillery on a larger scale, unless the average VS of a mixture is considered. Even then, distilleries could lose out on the maximum methane potential. The TS and VS of each stillage is dependent upon

parameters such as the grains used, ratio of grain to water in the mash bill, and even potentially the sampling of the stillage from the still. Some distilleries may strain water off, or process the stillage in other ways, also impacting these characteristics. The pH of the samples could be impacted by the yeast strain used, as this can impact the various by-products such as acids, and the age of the stillage. Often, the longer the stillage sits, the lower the pH will become. All efforts were made to sample the stillage fresh and load the BMPs with stillage as soon as possible after sampling for this reason.

An ANOVA was performed to determine if there was a significant difference in pH between the distilleries. The ANOVA produced a p-value of 0.4085, indicating there was no significant difference in the pH of the stillage across distilleries. The largest range within a distillery was Distillery F with a pH range of 0.59. In noticing the wide range of VS of stillage samples, an ANOVA for VS across distilleries was also performed, which produced a p-value of 0.0002, which indicates a significant difference in VS. Distillery G was identified from being significantly different from all other distilleries on the high end, while all others were not significantly different from at least two other distilleries (Table 4.5). The Duncan and Tukey tests provided slightly different results, but both had Distillery G as being significantly different from all other distilleries.

Table 4.4 In house tested stillage characteristics that aided in the loading of the BMPs where TS and VS are reported on a wet basis, and all values are reported as the mean ± standard deviation with n = 2.

Stillage	TS (% wb)	VS (% wb)	VS/TS (%)	pH
A1	5.7 ± 0.0	5.5 ± 0.0	95.16 ± 0.0	N/A
B1	4.5 ± 0.0	4.2 ± 0.0	93.60 ± 0.0	N/A
C1	5.73 ± 0.06	5.44 ± 0.05	94.99 ± 0.0	4.33
C2	7.61 ± 0.07	7.22 ± 0.07	94.77 ± 0.05	3.77
D1	5.42 ± 0.05	5.21 ± 0.02	96.05 ± 1.17	3.58
D2	7.60 ± 0.02	7.16 ± 0.02	94.18 ± 0.07	3.63
D3	6.62 ± 0.13	6.32 ± 0.13	95.52 ± 0.03	3.57
E1	8.69 ± 0.18	8.18 ± 0.17	94.14 ± 0.03	3.85
E2	8.71 ± 0.14	8.20 ± 0.15	94.13 ± 0.24	3.78
E3	8.26 ± 0.08	7.83 ± 0.08	94.72 ± 0.24	3.84
F1	4.82 ± 0.33	4.57 ± 0.33	94.85 ± 0.36	4.19
F2	4.92 ± 0.17	4.65 ± 0.16	94.67 ± 0.06	3.60
F3	6.06 ± 0.20	5.75 ± 0.20	94.84 ± 0.24	3.61
G1	15.37 ± 0.0	14.72 ± 0.02	95.73 ± 0.15	3.76

Table 4.5 Tukey results for an ANOVA of VS between distilleries, where the VS is represented as mean ± standard deviation for those with n > 1. Those with matching letters indicate a lack of a significant difference between VS.

% VS (wb)	n	Distillery
14.72^{A}	1	G
8.07 ± 0.2^{B}	3	E
$6.33 \pm 1.26^{B,C}$	2	C
$6.23 \pm 0.98^{B,C}$	3	D
$5.50^{B,C}$	1	A
$4.93 \pm 0.56^{B,C}$	3	F
4.20^{c}	1	B

For a better understanding of the macro nutrients of the stillage, the samples were sent to Regulatory Services at the University of Kentucky (Table 4.6). The macros present in the stillage represent potential nutrients for the microorganisms in the inoculum. The sole impact of the macronutrients in stillage are the grains utilized in the mash bills. An ANOVA for macronutrients produced no significant difference for protein, fat, fiber, and NDF with p-values greater than 0.05, but did produce significant differences for ADF across distilleries. For ADF, C was significantly different from G and D according to the Tukey test, and G and D were significantly different from all others according to the Duncan test. Although the p-value for the protein ANOVA was 0.08, the Duncan and Tukey tests identified significant differences between G and all others (Duncan) and G with F and C (Tukey).

Table 4.6 Macronutrients present in each stillage sample, with testing provided by Regulatory Services. Values are provided on a dry basis.

Stillage Sample	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	ADF (%)	NDF (%)
C1	27.09	10.01	7.48	11.75	29.60
C2	27.61	7.32	6.95	12.36	30.37
D1	24.00	9.45	9.01	21.50	39.31
D2	25.39	10.45	5.75	16.30	30.83
D3	28.25	13.44	5.82	17.46	34.71
E1	25.87	8.30	7.31	14.63	32.75
E2	26.85	8.76	6.85	15.51	26.07
E3	24.62	6.72	7.05	12.80	30.37
F1	28.62	9.68	8.05	12.51	32.54
F2	29.36	11.18	7.94	14.32	35.21
F3	25.37	6.18	7.02	13.73	34.49
G1	20.90	10.34	5.12	19.02	31.35

In performing linear regressions of the grain type (corn, rye, wheat, and barley) with macronutrient (protein, fat, fiber, ADF, and NDF) it was found that protein, fiber, ADF, or NDF content did not have a direct linear correlation with grain content. Meanwhile, fat content has a positive correlation with corn content with an overall model p-value of 0.0008, R^2 of 0.73, and parameter estimates of:

$$y_{fat} = 0.088x_{corn} + 3.90$$

With p-values of 0.0008 and 0.0071 respectively, showing the significance of the fit of the line. The Cook's D analysis does provide two outliers, samples D3 and F3. Sample D3 contained the highest amount of fat, at 13.44% while F3 contained the least amount of fat with 6.18%. Since the mash bill for D3 was an estimate, it is possible that it contains more or less corn than assumed. Along with this concept, rye has a negative correlation with fat content, with a p-value of 0.0122 but an R^2 of only 0.52. This relates to the idea of more corn correlates to more fat because the higher the corn concentration, the less rye that is used. Barley and wheat did not have a well fit linear regression with fat content. Although an R^2 closer to 1 is preferred, the R^2 of 0.7645 for corn and 0.7789 for barley signifies that mash bill does have an impact on the macronutrient content of stillage, but only accounts for three-fourths of the variance.

While minerals can provide energy sources for microorganisms, they also present themselves as inhibitors in some cases. Through the feed services at Regulatory Services, mineral compositions of each stillage sample were provided (Table 4.6). Copper (Cu) is commonly known as an antimicrobial; stillages C1, C2, and G1 had higher concentrations than the others, which could contribute to lower methane yields. In distillation, stills are lined with copper, likely leading to the copper in the stillage. Sample C1, which was produced in the larger of the columns at Distillery C, contained the greatest amount of copper. The only sample produced in a pot still, G1, contained the next highest amount of copper, likely because the stillage rests in the bottom of the pot still, and does not flow through. As indicated by Distillery G, the distillation of 175 – 200 gallons takes five to six hours, and during that time, the stillage stays sedentary at the bottom of the pot. Another noticeable difference in mineral composition was the iron (Fe) concentration found in sample F3, at 1152 ppm. Previous studies indicate that the addition of iron in low amounts can be beneficial to digestion, but in higher amounts, the process can become hindered.

Table 4.7 Mineral composition of stillage samples from Regulatory Services. All values are provided on a dry basis.

	Ca (%)	P (%)	Na (%)	K (%)	Mg (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
C1	0.12	0.83	0.04	1.01	0.27	142.00	131.00	36.80	53.70
C2	0.12	0.72	0.03	0.97	0.25	49.70	297.00	41.00	60.90
D1	0.07	0.86	0.03	0.97	0.27	10.30	196.00	21.30	73.30
D2	0.09	0.97	0.03	1.16	0.34	9.83	223.00	30.00	64.60
D3	0.08	0.81	0.02	0.90	0.29	7.91	433.00	20.50	75.70
E1	0.08	0.99	0.01	1.10	0.31	9.67	166.00	0.31	87.50
E2	0.06	0.95	0.01	1.09	0.34	6.99	185.00	32.40	48.60
E3	0.10	0.89	0.01	1.26	0.33	4.61	378.00	38.80	76.10
F1	0.11	0.84	0.03	1.00	0.30	11.60	167.00	20.40	57.80
F2	0.11	0.83	0.06	0.90	0.35	10.60	527.00	15.00	72.95
F3	0.13	0.83	0.04	1.15	0.29	15.90	1152.00	42.70	128.00
G1	0.09	0.72	0.05	0.82	0.25	83.80	72.10	21.70	40.35

Since rye and corn are high in iron, it could be assumed that the iron concentration in the stillage likely stemmed from them. Given the variation in iron in

stillage, a linear regression was performed to determine the influence of corn and rye on iron content. The model produced a p-value of 0.04 but with a low R^2 of 0.39. Despite the low R^2, the model is still indicative of a negative relationship between corn and iron, seeing as the corn concentration in the stillage increases, iron concentration decreases. With the 95% confidence interval, F2 was considered an outlier. As for a regression with rye, a p-value of 0.02 indicates a relationship between rye and iron, despite the low R^2 of 0.47, which happens to be a positive relationship.

One of the most important qualities to consider when developing a BMP is the carbon and nitrogen concentrations, along with the ratio of carbon to nitrogen. The ideal C/N for anaerobic digestion is 25 – 30, where the stillage samples were half to one-third of that (Table 4.7). The values for Carbon and Nitrogen were provided on a wet basis, but each sample was partially dried to a low moisture content, creating a minimal difference in the values on a wet and dry basis. The lower C/N placed the reactors at risk of failure due to Nitrogen, but after measuring the total ammonia-nitrogen (TAN) at the end of the BMP, this was determined to be a non-issue, as the reactors did not have any ammonia inhibition.

Table 4.8 Carbon and Nitrogen content of each stillage sample provided on a wet basis with n = 3 and values presented as mean ± standard deviation.

Stillage	Carbon (%)	Nitrogen (%)	C/N
D1	49.58 ± 0.03	5.44 ± 0.03	9.12 ± 0.05
D2	47.84 ± 0.08	4.6 ± 0.22	10.42 ± 0.53
D3	49.51 ± 0.15	5.71 ± 0.10	8.67 ± 0.15
E1	48.37 ± 0.15	5.74 ± 0.13	8.42 ± 0.18
E2	47.73 ± 0.01	4.83 ± 0.13	9.88 ± 0.26
E3	45.95 ± 0.06	4.45 ± 0.06	10.33 ± 0.13
F1	48.39 ± 0.20	4.54 ± 0.07	10.65 ± 0.16
F2	49.05 ± 0.23	5.06 ± 0.13	9.70 ± 0.25
F3	46.02 ± 0.13	4.72 ± 0.05	9.75 ± 0.09
G1	47.28 ± 0.38	4.43 ± 0.44	10.76 ± 1.00

4.1.2 Pre- BMP Characterization

At the startup of the BMP, TS, VS, pH, and alkalinity were tested (Table 4.7). The testing of TS and VS was beneficial for determining if the BMP was loaded correctly and to determine the VS destruction at the end of the 28-day BMP. Since pH is a critical factor in BMP success, measuring the pH allows for the assurance that the BMP will be operating under near ideal conditions, and the alkalinity provides information on the likelihood of failure due to VFA accumulation. The higher the alkalinity, the more acid that is required to reach a pH of 4.5, meaning the BMP is less likely to fail due to VFA accumulations.

Table 4.9 Pre-BMP characteristics where each sample was the mixture of stillage, inoculum, and water based on the loading criteria calculated from initial stillage characteristics. TS and VS values are provided on a wet basis, and all values are represented as the mean ± standard deviation and n = 3. Alkalinity does not possess a standard deviation because it was measured with composite samples.

Stillage	% TS	% VS	% VS/TS	pH	Alkalinity (mg $CaCO_3$/L)
C1	1.18 ± 0.22	0.79 ± .019	66.21 ± 4.99	7.68 ± 0.02	N/A
C2	1.84 ± 0.20	1.40 ± 0.19	76.00 ± 2.15	7.59 ± 0.07	N/A
D1	4.43 ± 0.01	2.82 ± 0.01	65.89 ± 0.15	7.12 ± 0.01	3670.0
D2	5.09 ± 0.16	3.24 ± 0.08	63.71 ± 0.48	6.96 ± 0.08	4108.3
D3	5.25 ± 0.56	3.21 ± 0.26	61.29 ± 1.58	6.99 ± 0.09	3191.5
E1	4.58 ± 0.02	3.00 ± 0.01	65.49 ± 0.10	7.27 ± 0.02	4187.5
E2	4.65 ± 0.03	2.99 ± 0.20	64.27 ± 0.18	7.22 ± 0.09	3348.3
E3	4.92 ± 0.20	3.07 ± 0.10	62.32 ± 0.59	7.03 ± 0.11	3867.7
F1	4.68 ± 0.60	2.92 ± 0.52	62.05 ± 0.48	7.43 ± 0.07	3938.8
F2	4.85 ± 0.13	3.13 ± 0.06	64.44 ± 0.70	7.11 ± 0.04	3240.0
F3	4.92 ± 0.36	3.10 ± 0.17	63.20 ± 1.32	6.81 ± 0.01	3351.4
G1	4.97 ± 0.10	3.21 ± 0.09	64.60 ± 0.52	7.18 ± 0.06	3299.4

Since the inoculum used in each BMP was acquired from the BMP before it, the TS and VS of the inoculum varied slightly with each BMP (Table 4.8). Since stillage samples C1 and C2 were performed in Phase One, and D1 and E1 were performed first in Phase Two, the initially acquired inoculum was used. Despite the differences in TS and VS of the stillage, each BMP was loaded with the same desired FM and OLR where the amount of VS was consistent across each one.

Table 4.10 TS and VS of the inoculum used in each BMP, which varied based on the recovery and centrifugation of the digestate from the previous BMP. Samples in the first three BMPs were calculated with n=2, then increased to n=3 for the last two BMPs as the heterogeneity of the inoculum increased. All values are represented as the mean ± standard deviation.

Samples in BMP	% TS	% VS	% TS/VS
C1, C2	5.95 ± 0.06	3.39 ± 0.05	57.08 ± 0.26
D1, E1	4.17 ± 0.03	2.37 ± 0.02	56.93 ± 0.05
D2, E2, F1	10.11 ± 0.0	5.75 ± 0.0	56.82 ± 0.0
F2, G1	12.67 ± 0.24	7.07 ± 0.06	55.84 ± 1.22
D3, E3, F3	12.82 ± 0.51	7.03 ± 0.27	54.85 ± 0.13

4.1.3 Post-BMP Characterization

At the end of the 28-day BMP, the same tests were performed as at the beginning of the BMP, only total ammonia-nitrogen (TAN) replaced alkalinity (Table 4.9). Measuring the VS at the end of the BMP allowed for the determination of VS destruction for each sample. Checking the pH and TAN levels indicated if a possible failure occurred either due to VFA accumulation, which would lower the pH, or TAN inhibition, which typically occurs at higher pH. Since ammonia toxicity occurs at 1500 – 3000 ppm at a pH of 7.4 [12], it is clear the none of the samples experienced ammonia inhibition. Although the pH for each was above 7.4, the TAN concentration was approximately half that of the toxicity level for samples D – G. It is evident that VFA accumulation did not cause any failures since the final pH of all samples were between 7.30 and 7.86. The only caveat is for samples C1 and C2, where midway through, methane accumulation had reduced. In checking the pH, it was determined the pH had decreased, and a buffer of sodium bicarbonate was required to mitigate the issue. Without the buffering, the final pH would have been lower, likely around 6.5, as that was the pH measured at the first notice of BMP failure. Samples D3, E3, and F3 are all listed as approximate TS and VS values due to an error in method where the weight of the aluminum weigh boat was not recorded and instead had to be estimated to complete the calculations.

Table 4.11 Post-BMP characteristics from each sample at the end of the 28 day BMP to determine VS destruction or if there was inhibition due to VFA accumulation or ammonia inhibition. TS and VS values are provided on a wet basis, and all values are represented as mean ± standard deviation with n = 3.

Stillage	TS (% wb)	VS (% wb)	VS/TS (%)	pH	TAN (ppm)
C1	1.30 ± 0.06	0.55 ± 0.01	42.32 ± 1.05	7.77 ± 0.04	1189
C2	1.29 ± 0.03	0.57 ± 0.01	44.65 ± 0.73	7.77 ± 0.01	1270
D1	3.51 ± 0.02	1.96 ± 0.01	55.86 ± 0.23	7.71 ± 0.01	823
D2	4.13 ± 0.21	2.21 ± 0.12	53.48 ± 0.17	7.57 ± 0.03	806
D3*	3.79 ± 0.08	2.04 ± 0.02	53.73 ± 1.51	7.43 ± 0.02	809
E1	3.65 ± 0.04	2.04 ± 0.02	56.06 ± 0.34	7.86 ± 0.01	817
E2	3.83 ± 0.03	2.06 ± 0.01	53.66 ± 0.29	7.46 ± 0.04	824
E3*	3.96 ± 0.03	2.08 ± 0.04	52.55 ± 1.22	7.30 ± 0.07	793
F1	3.94 ± 0.17	2.12 ± 0.08	53.68 ± 0.59	7.46 ± 0.06	837
F2	3.78 ± 0.07	2.03 ± 0.04	53.66 ± 0.21	7.69 ± 0.05	824
F3*	3.99 ± 0.09	2.20 ± 0.05	55.27 ± 0.95	7.43 ± 0.05	754
G1	3.87 ± 0.13	2.09 ± 0.05	54.11 ± 0.47	7.49 ± 0.06	836

*Approximate TS and VS values

While stillage is not a good candidate for use as a fertilizer, the digestate for AD is. Fertilizers are measured by the amount of Nitrogen, Phosphorus, and Potassium present, but it is also important to understand the content of various other minerals (Table 4.12). Minerals such as Zinc, Copper, and Manganese appeared in lower concentrations, while Calcium were in slightly higher concentrations. Fertilizer grade was determined from this information, with the assumption that Nitrogen is derived from 50% of the available Nitrogen, Phosphorus was derived from 80% of the available P_2O_5, and Potassium from 100% of the K_2O available (Table 4.13). The fertilizer grade was estimated based on the concentrations of those products in pounds per 1000 gallons (lbs/1000 gal). All digestate samples contained high Phosphorus levels, making it a high Phosphorus fertilizer with low Potassium levels.

Table 4.12 Liquid manure analysis results of the digestate derived from composite samples with n = 3. Values are in g/L unless otherwise noted.

	D1	D2	D3	E1	E2	E3	F1	F2	F3	G1
N	75.1	34.7	45.7	75.5	31.9	46.3	54.7	51.2	43.8	16.4
P_2O_5	119.5	66.2	95.9	118.2	58.0	102.3	99.4	102.3	102.3	33.5
K_2O	23.9	9.5	11.4	26.3	5.80	12.2	9.94	19.5	12.2	3.9

Ca	30.7	15.8	22.8	29.6	15.9	24.4	24.9	24.4	26.8	7.8
Mg	6.8	3.2	4.6	6.6	4.4	4.9	4.97	7.3	4.9	2.3
Zn	0.9	0.5	0.9	0.9	1.0	0.9	0.8	0.8	1.3	0.3
Cu	0.27	0.08	0.14	0.26	0.03	0.17	0.15	0.24	0.17	0.06
Mn	0.8	0.8	0.7	0.8	0.7	0.7	1.2	0.9	0.7	0.3
TC (%)	33.1	17.1	22.5	1.2	14.7	23.6	25.5	24.2	25.2	8.3
TN (%)	7.6	3.4	4.6	7.5	3.2	4.5	5.4	5.1	4.5	1.6

Table 4.13 Fertilizer grade values of each stillage sample based on the amount of N - P - K available from each in lbs/1000 gallons.

Stillage Sample	Fertilizer Grade N-P-K
D1	13 – 34 – 8
D2	13 – 40 – 7
D3	12 – 40 – 6
E1	14 – 35 – 1
E2	13 – 38 – 5
E3	11 – 40 – 6
F1	13 – 38 – 5
F2	12 – 40 – 10
F3	11 – 40 – 6
G1	13 – 41 – 6

4.2 Biomethane Potential of Stillage

4.2.1 Phase One BMPs

In the starting phase of this experiment, an OLR of 5 g VS/L and FM of 1 g VS/g VS was utilized, which proved to cause some issues. Stillage samples A1 and B1 were analyzed in one BMP, while C1 and C2 were analyzed together in another. Notably, Stillage B1 had a higher methane yield, with A1 not far behind (Figure 4.3). During the BMP with stillages C1 and C2, it was noticed that production was slowed or stopped in some instances, beginning on day 7. For two samples of C2 and one sample of C1, the methane production has ceased for over 48 hours. Upon noticing this, pH was checked, and the pH was low, with a pH of 6.25 for C2 samples and 6.46 for C1. To combat the likely accumulation of VFAs, 15 mL for C1 and 20 mL for C2 of a 1M solution of sodium bicarbonate was used as a buffer. The buffer increased the pH to 6.86 (C2) and 6.94 (C1). Each instance the pH was noticed to be low, 10 to 20 mL of the buffer was

added, depending on the pH. While this seemed to periodically increase the pH closer to neutral, it is likely that the methanogens were already harmed due to the VFA accumulation, leading to the overall decreased methane production. The addition of sodium bicarbonate to the system is not ideal in terms of cost and impact on the composition of the system, leading to a need to mitigate this issue.

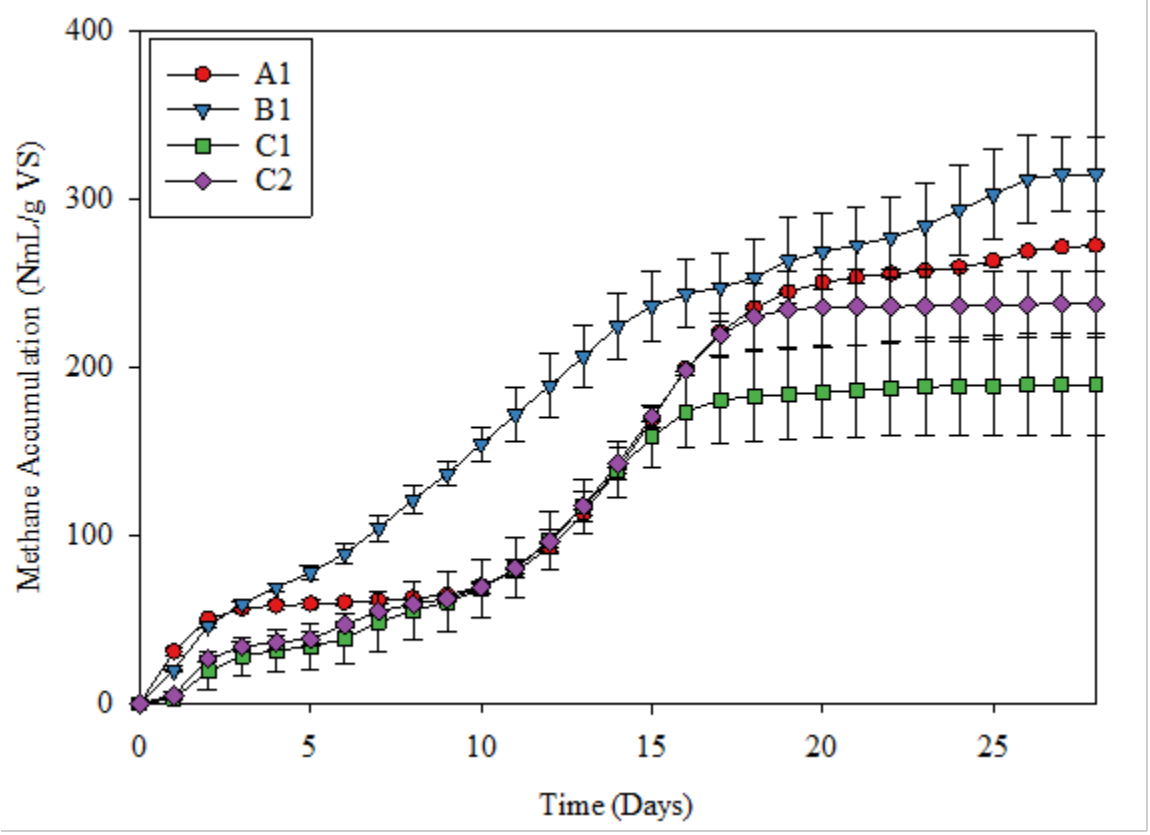

Figure 4.3 Results of the Phase One BMPs over the course of 28 days with data points representing the mean and error bars for standard deviation where n = 2 for A1 and B1 and n = 3 for C1 and C2

Although the methane accumulation of A1 and B1 appear similar, and B1 and C1, statistical analysis was performed to determine if there is a statistical significance between methane production for each stillage sample. Comparing C1 and C2 provides insight on the impact of mash bill, as they were acquired from the same distillery. An ANOVA at the significance level of 0.05 produces a p-value of 0.0046, indicating there is a statistically significant difference between the methane production of one or more of the stillage samples. From the Tukey test, it was determined that there is not a significant

difference between stillages A1 and B1, A1 and C2, and C2 and C1 (Table 4.14). The lack of a significant difference between C1 and C2 shows little impact due to the variation in mash bill, as they were produced by the same distillery. Both the Duncan and Tukey tests showed the same grouping of mash bills based on the methane accumulation.

Table 4.14 Tukey grouping for Phase One BMPs where those with the same letter(s) indicate a lack of a significant difference in the mean methane accumulation. Methane value is represented as the mean ± standard deviation.

Mean Methane Accumulation (NmL/g VS)	n	Mash Bill
314.73 ± 22.03^{A}	2	B1
$272.50 \pm 0.92^{A,B}$	2	A1
$237.78 \pm 19.45^{B,C}$	3	C2
189.65 ± 30.40^{C}	3	C1

Although phase one BMPs did not produce high yields of methane, it provided some insight to the optimization of the AD of stillage. The methane yields of these samples were as low as 38% of those found in literature, up to 63%, proving the yields could be higher. Interestingly, the results contradicted the initial prediction of the physicochemical characteristics and distillation parameters significantly impacting the BMP of the stillage. With so few samples though, it is hard to determine if this is the case across the board with distilleries in Kentucky. Phase two tests 10 different stillages, providing slightly better insight to the impact of mash bill and distillation parameters on the BMP.

4.2.2 Optimization of BMPs

Although conclusions could be made from the data produced in phase one, the BMP was not stable, and the methane yields were low in comparison to those found in literature and other successful BMPs within other industries. Using the resources available, with A1 being the most available stillage at the time, various parameters were tested for the BMP. The plan was to test OLRs of 5 and 10, but due to the low FM combined with the high OLR, the FM of 0.25 required an OLR of 7.5 to stay within the working volume of 400 mL. By day 25, it was evident which parameters would work the best, therefore the BMP ended early. The FM of 0.25 with the OLR of 7.5 had the highest methane yield, with the FM of 1 and OLR of 5 having the lowest methane yield (Figure

4.4). The lack of production from the FM 1 and OLR 5 was likely due to the increased storage time of the stillage prior to use, as it was the remaining stillage from the first BMP where A1 was used. Over time, the pH of stillage continuously drops below the already acidic pH level.

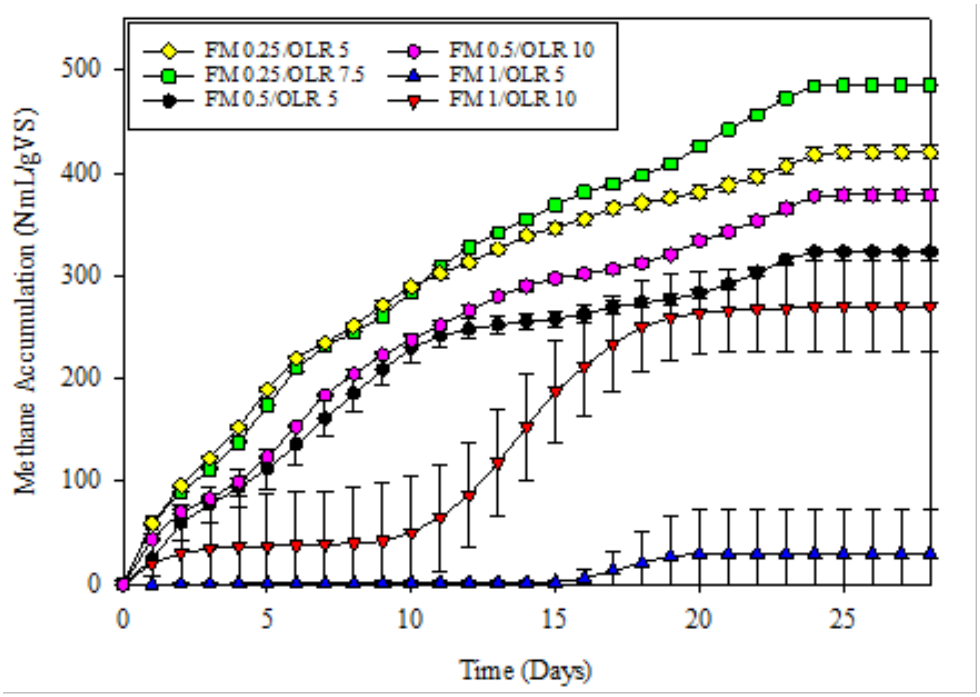

Figure 4.4 Daily methane accumulation for parameter optimization samples for a BMP of 25 days where n = 2 and the data points represent the mean with error bars of standard deviation. Parameters of FM 0.5 g VS/g VS and OLR of 10 were chosen based on these results.

In performing an ANOVA for each parameter test at the 0.05 significance level, a p-value of near 0 is produced, signifying highly significant differences between the parameter tests, as expected based on the raw data. Tukey grouping identified where the significant differences occurred in the methane production for each FM and OLR combination (Table 4.15) The FM of 1 with an OLR of 5 was significantly difference than all other parameters, but one of the duplicates did not produce any gas, contributing to the low mean accumulation and high standard deviation. Based on the data presented, the FM of 0.5 with an OLR of 10 was chosen, seeing as it allows for a much higher yield than the FM of 1 with an OLR of 5, and is much more feasible in terms of resources with

an inoculum than those with the FM of 0.25. Seeing as there appears to be no significant difference from the FM of 0.25 with an OLR of 5 according to the Tukey test, this seemed like a reasonable assumption to make moving forward.

Table 4.15 Tukey test results for parameter optimization tests where those with the same letter(s) indicate a lack of a significant difference in the mean methane accumulation.

Mean Methane Accumulation (NmL/g VS)	n	FM	OLR
485.68 ± 0.78^{A}	2	0.25	7.5
$420.55 \pm 7.21^{A,B}$	2	0.25	5
378.89 ± 5.07^{B}	2	0.5	10
$323.45 \pm 2.62^{B,C}$	2	0.5	5
270.56 ± 45.02^{C}	2	1	10
29.83 ± 42.18^{D}	2	1	5

4.2.3 Phase Two BMPs

When the parameters of the BMP were changed to the FM of 0.5 g VS/g VS and an OLR of 10 g VS/L, 10 more stillage samples were tested. All samples were tested in triplicate, but for samples D2, E2, and F2 only two of the three were considered for methane accumulation in the BMP due to a failure in the gas absorption unit for one replication of each stillage sample. In these BMPs, sample E3 produced the least amount of methane at 291.71 ± 3.45 NmL/g VS while D2 produced the most at 419.19 ± 2.62 NmL/g VS (Figure 4.5). The highest producer, D2, was a wheat bourbon stillage while E3 was a rye whiskey with 51% rye. Apart from E3 and F3, all samples followed a similar kinetics pattern, and ended with very similar methane accumulations. For a better look at how the methane accumulations compared for each sample, a boxplot was created to look at the distribution for each stillage sample (Figure 4.6). With the boxplot, it became evident which stillage samples produced the most, whereas in the daily accumulation graph, many samples overlapped, making it difficult to determine which sample was the highest producer. The boxplot also provided better insight to with samples produced the higher standard deviations, and which ones had minimal deviation.

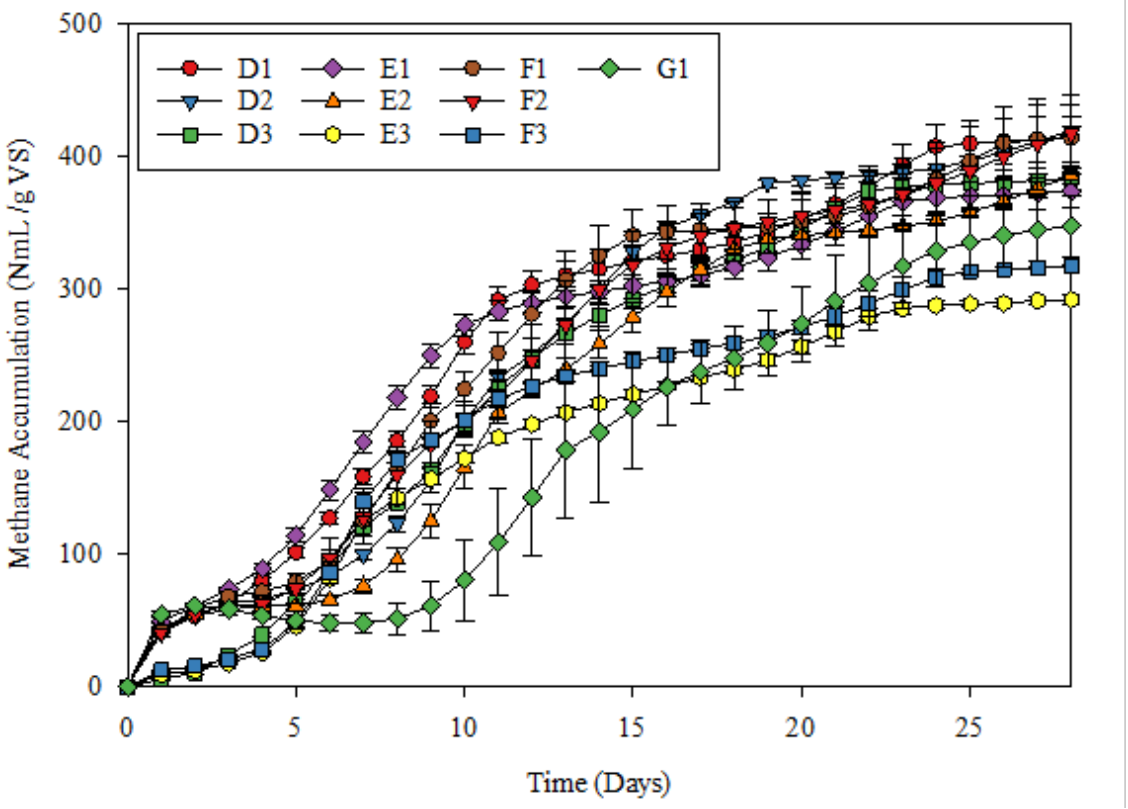

Figure 4.5 Daily methane accumulation for Phase Two BMPs where the data points represent the mean with error bars for standard deviation and n = 3 (except for D2, E2, and F1 where n = 2).

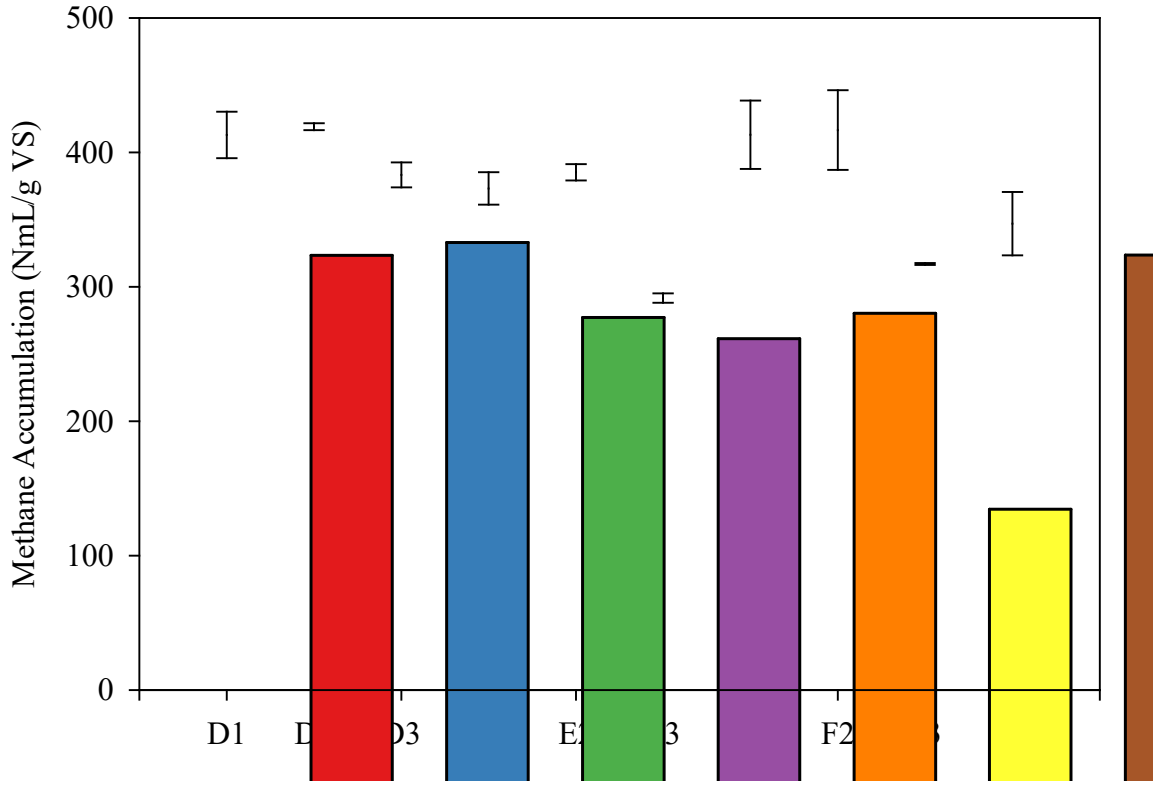

Figure 4.6 Bar charts of final methane accumulation for Phase Two BMPs where n = 3 except for D2, E2, and F1 where n = 2, and all values are the mean with error bars as standard deviation.

Overall, there seems to be significant differences in methane accumulation between each of the stillage samples. With an ANOVA at an α level of 0.05, a p-value of near 0 (less than 0.0001) was achieved, indicating there is a significant difference in the mean methane production of at least one sample. For determining this difference, a Tukey tests was performed (Table 4.16). The Tukey groupings indicated that samples E3, F3, and G1 were significantly different from all other samples, except for no F3 which was not significant different from G1 but was also not significantly different from all others that produced higher amounts of methane.

Table 4.16 Tukey groupings for Phase Two BMPs. Mash bill samples with matching letters indicate a lack of a significant difference in methane production. Methane accumulation is reported in mean ± standard deviation.

Methane Accumulation (NmL/g VS)	n	Mash Bill
419.19 ± 2.62^{A}	2	D2
416.68 ± 29.65^{A}	3	F2
413.20 ± 25.40^{A}	2	F1
413.06 ± 17.32^{A}	3	D1
$385.33 \pm 6.06^{A,B}$	2	E2
$383.31 \pm 9.28^{A,B}$	3	D3
$373.28 \pm 12.11^{A,B}$	3	E1
$347.12 \pm 23.59^{B,C}$	3	G1
$317.05 \pm 0.73^{C,D}$	3	F3
291.71 ± 3.45^{D}	3	E3

In analyzing the Tukey test, we can identify where significant differences occur within the distilleries, indicating an impact of mash bill on the BMP. An ANOVA was also performed for each mash bill within the distilleries to determine If significant differences occurred or not. For distillery D, a p-value of 0.042, showing a small significant difference, shown to be a difference between D2 and D3 (Table 4.17a). Distillery E produced a much lower p-value, <0.0001, where the Tukey test indicated a lack of a significant difference between E1 and E2, but both were significantly different from E3 (Table 4.17b). Similar to Distillery E, Distillery F produced a low p-value (0.005), Tukey indicating a significant difference of F1 and F2 from F3 (Table 4.17c). For each distillery, the sample that has a significant difference between the other two samples, was operated in the same BMP. Although the data indicates a difference in the methane production based on the mash bills, is it possible that something within the BMP caused lower yields. In comparing D3 to E3 and F3, D3 did produce a significantly higher yield (Table 4.16). Both E3 and F3 stillages were those higher in rye, indicating rye could have some impact to the lower methane yields.

Table 4.17 Tukey grouping for Distillery D (a), Distillery E (b), and Distillery F (c). Matching letters within each grouping indicates the lack of a significant difference in the methane accumulation.

Methane Accumulation (NmL/g VS)	Mash Bill
Table 4.17a	
419.19 ± 2.62^{A}	D2
413.06 ± 17.32^{A,B}	D1
383.31 ± 9.28^{B}	D3
Table 4.17b	
385.33 ± 6.06^{A}	E2
373.28 ± 12.11^{A}	E1
291.71 ± 3.45^{B}	E3
Table 4.17c	
416.68 ± 29.65^{A}	F2
413.20 ± 25.40^{A}	F1
317.05 ± 0.73^{B}	F3

Since most samples contained corn, rye, and barley, analyses were performed to determine the impact of corn, rye, and barley concentrations on the methane yield. Wheat was not considered in the linear regressions because in Phase Two there was only one sample that contained wheat, and the mash bill data was not available for that. Given the lack of variety of barley present in the mash bills, there was not a significant linear relationship between barley concentration and methane production. There does appear to be a positive linear relationship of methane with corn and rye (Figure 4.7). Corn has a positive linear relationship with methane, with a p-value of 0.02, but with a low R^2 of 0.58, accounting for approximately half of the variance. With a larger sample variety, this could change. The estimated relationship of corn and methane can be represented by:

$$y_{methane} = 1.589 x corn + 272.07$$

Considering as corn content increases, rye content decreases, rye and methane have a negative linear relationship. The negative relationship seems reasonable since rye has been considered an antimicrobial in the past. With a p-value of 0.03, but a low R^2 of 0.51, the estimated relationship of rye and methane can be represented by:

$$y_{methane} = -1.37 x_{rye} + 398.64$$

Although both models produced low R^2 values, there does appear to be a linear relationship between corn and rye with methane given the low p-values.

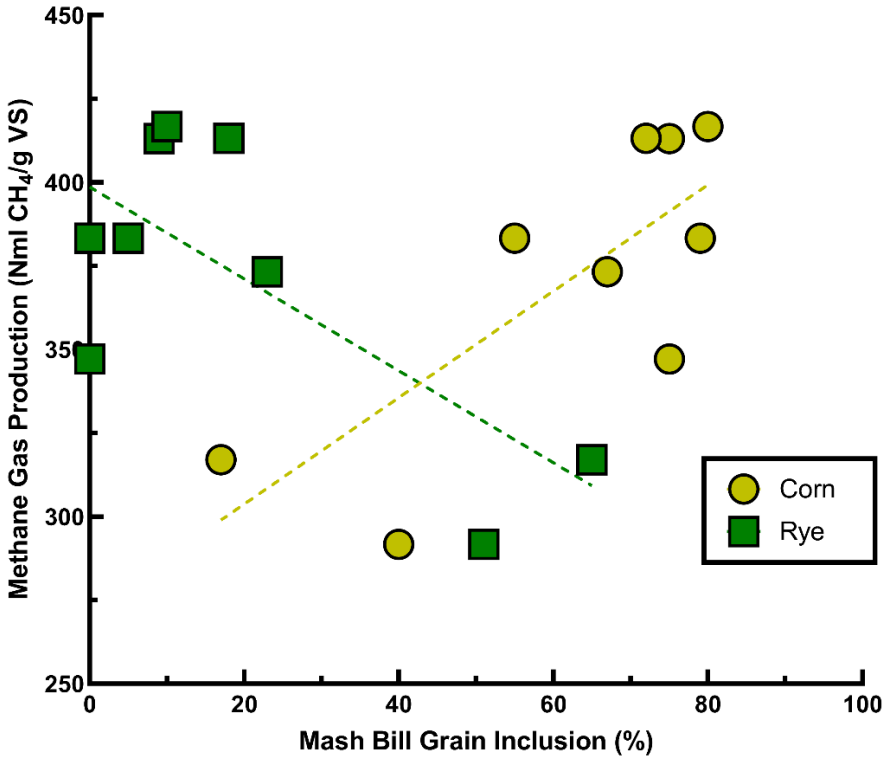

Figure 4.7 Linear relationship of methane from corn and rye, with a positive relationship with corn and a negative relationship with rye. This only accounts for samples from distilleries D through G, except for D2 where no information on the mash bill was provided.

Since the grains and distillation parameters impact characteristics of the stillage, especially the heavy metals and macronutrients, linear regressions were also performed on heavy metals (Copper, Iron, Manganese, and Zinc) and the macro-nutrients (Protein, Fat, Fiber) to determine if they positively or negatively impact methane production. Copper is known to be an inhibitor of AD, and the linear regression for copper proves this with a p-value of 0.008, although the R^2 is only 0.53:

$$y_{methane} = -1.30x_{Cu} + 388.20$$

Manganese presents a negative linear relationship as well, but the R^2 is much lower at 0.37, likely considering the addition of Manganese at some levels improves AD,

and at higher levels it seems to inhibit. Iron has no linear relationship with methane production, nor does zinc. With more data, these relationships could likely change. The data was not perfectly normal, but the assumption of normality was made for the sake of the linear regressions. The regressions performed for the macro-nutrients also lacked indication of a linear relationship with methane production.

4.2.4 Kinetic Modeling

The kinetics modeling of the BMP was performed using a modified Gompertz model. This model produced high R^2 values, with the lowest being 0.94 for sample G1 (Table 4.18). When analyzing sample G1, it was noticed that there was a very distinct secondary lag phase, which was not accounted for in the model. Instead, the model shows a steady increase in methane production at that time. The Gompertz model appears to be an overestimate for samples D1, D2, D3, E2, F2, and G1, but an underestimate for E1, E3, F1, and F3. Given the high R^2 values, it can be assumed that this model accounts for a high amount of the variance present. The root mean square errors (RMSEs) are slightly high, indicating that the model does not perfectly estimate methane accumulation. This could potentially be reduced by introducing more replicates into the experiments, or by finding a two-phase Gompertz model to fit the data with, seeing as most samples contain a secondary lag phase.

Table 4.18 Results of the Gompertz model using n = 3 (except for D2, E2, and F1 where n = 2). The Gompertz model presents itself as a viable modeling tool for estimating the BMP.

Mash Bill	P (mL CH_4/g VS)	R_m (mL CH_4/g VS - d)	Lamda (days)	R^2	RMSE
D1	409.9	26.5	0.8	0.98	17.93
D2	431.6	27.1	2.5	0.99	15.03
D3	393.2	26.7	2.8	1.00	8.316
E1	363.8	27.1	0.3	0.98	15.77
E2	398.1	23.3	2.6	0.98	16.87
E3	288.6	20.7	2.1	0.99	11.94
F1	407.9	27.0	1.6	0.98	20.21
F2	419.3	24.4	1.6	0.98	20.99
F3	300.6	25.4	2.3	0.98	15.35
G1	446.2	17.2	3.6	0.94	29.64

The parameters from Table 4.17 were then used to estimate the expected values given the Gompertz model equation $y = Pe^{-e^{(\frac{R_m e}{P}(\lambda - t)+1)}}$ and inputting the time in days (Figure 4.7). The actual values are represented by the datapoints, while the expected values are represented by the solid line. The model does not account for any additional lag phases beyond the initial one, which often causes an over or underestimation of the expected values. The graphical analysis continued the BMP data up to day 40, even though the actual BMPs were carried out for 28 days. This shows that beyond day 28, the BMPs would likely not produce a significant amount of additional biogas to justify increasing the retention time. Sample D1 had an additional lag phase beginning around day 10 and ending around day 18, which was not accounted for in the model (Figure 4.7a). A similar situation occurred with sample E2, where the lag phase from approximately day two to day seven was not accounted for in the Gompertz model, leading to a slight overestimation (Figure 4.7c). Sample F1 also had a strange lag in production, as did G1, which seemed to slightly increase in the daily methane production towards the end of the BMP (Figure 4.7d). Sample G1 had the largest overestimation, but this is likely because the methane production did not plateau towards the end, but rather seemed to begin steadily increase. Although the Gompertz model provides a great starting point for a model, other models could potentially better fit the data, and can provide larger overestimations as it did for G1.

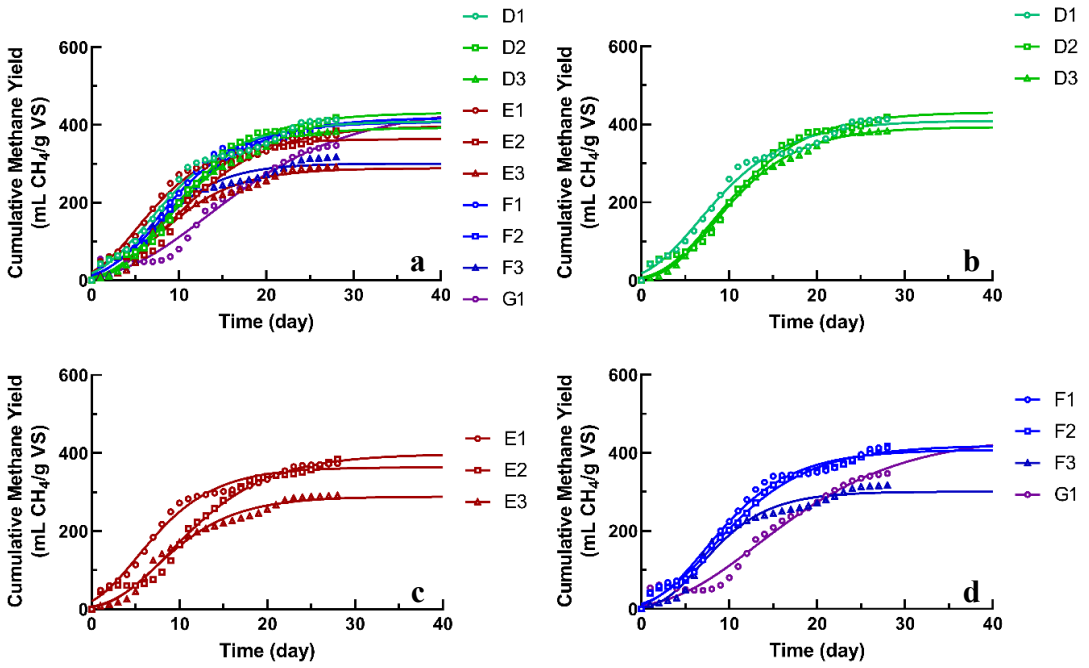

Figure 4.8 Comparison of the Gompertz model (solid line) and actual data (data points) from the GraphPad Prism analysis of (a) all samples, (b) samples from Distillery D, (c) Samples from Distillery E, and (d) samples from Distilleries F and G.

4.2.5 Analysis of Energy

For determining the potential energy output of AD of stillage at a distillery, it was assumed that the distillery produces equal amounts of each stillage type, methane produces 10.45 kWh/m^3, and the energy conversion efficiency of combined heat and power (CHP) is 35%. Using these assumptions, and the average cost of energy at the industrial level as 7.07 cents/kWh, the estimated amount of electricity and dollar value of electricity potentially produced by the distilleries was estimated (Table 4.19). Distillery C's values are on the low end of the spectrum, considering the BMP occurred during Phase One under different parameters that were not optimal. Distillery E did not provide data pertaining to the annual stillage production, leading to an assumption that they produce similar amounts as Distillery D, given they are both larger distilleries within the state. Currently, distillery G produces approximately 190,000 gallons of stillage annually, stemming from 19,875 proof gallons or 300 barrels. An expansion is underway with the

intent to produce 100 barrels a day (at the assumption of the production only 300 days of the year), equating to a little over 1.5 million gallons of bourbon, hence the 15 million gallons of stillage produced annually.

Table 4.19 Estimated financial outcome for distilleries performing AD on stillage. Distillery E did not provide stillage data, but an assumption was made based on the size of the company.

Distillery	Annual Stillage Production (M gal)	Annual Energy Production (MWh)	Value of Electricity ($)
C	29	5,519	390,164
D	100	34,969	2,472,302
E[a]	100	39,202	2,771,536
F	12.8	3,304	233,577
G[b]	0.19	134	9,504
G[c]	15	10,613	750,350

a: estimated stillage production
b: current production
c: estimated production based on upcoming distillery expansion

Between the five distilleries, approximately 250 million gallons of stillage is produced annually, and with the current industry expansion, this will likely double within the next five years. Distillery F indicated that the 12.8 million gallons of stillage is not indicative of the amount they use for sour mashing, which accounts for approximately 13-20% of their stillage. With that, they produce around 14.7-16 million gallons of stillage from approximately 2.2 million proof gallons, which is the amount of product removed from the still. Distillery F did not include the 13-20% used in sour mashing because to them, that is not considered the waste product, as it is reused in the next distillation. Distillery C indicated spending approximately $8,000 per day to dispose of stillage to farmers. If an assumption is made that stillage is transported approximately 300 days out of the year, the distillery is spending $2.4M annually, and with AD this cost would turn into $390K income, likely more because the stillage from Distillery C did not approach biomethane potential. At current production rates, AD does not seem feasible for stillage usage for Distillery G, seeing as the value is approximately $10,000 annually, but could pose as an option when the distillery expands. Along with performing AD comes capital costs to purchase and maintain the equipment, which would alter the financial impact of deciding to implement AD.

CHAPTER 5. CONCLUSION AND FUTURE WORK

After the analysis of methane production from the AD of stillage, AD does present itself as a viable option for stillage valorization, with the potential to have a positive financial impact. Based on the results presented, distillation parameters and mash bill do appear to impact the BMP of the stillage. Methane accumulation ranged from 189.65 ± 30.40 NmL/g VS to 314.73 ± 22.03 NmL/g VS with an FM of 1 g VS/g VS and an OLR of 5 g VS/L and 291.17 ± 3.45 NmL/g VS to 419.19 ± 2.61 NmL/g VS with an FM of 0.5 g VS/g VS and an OLR of 10 g VS/L (Table 5.1). The distillation parameters impact qualities such as the minerals found in the stillage, while the individual grains in the mash bill have an impact on the minerals and macronutrients of the stillage.

Table 5.1 Overall results for all stillage samples with the methane accumulation represented as the mean ± standard deviation.

Mash Bill	FM (g VS/g VS)	OLR (g VS/L)	n	Methane Accumulation (NmL/g VS)
A1	1	5	2	272.50 ± 0.92
B1	1	5	2	314.73 ± 22.03
C1	1	5	3	189.65 ± 30.40
C2	1	5	3	237.78 ± 19.45
D1	0.5	10	3	413.06 ± 17.32
D2	0.5	10	2	419.19 ± 2.62
D3	0.5	10	3	383.31 ± 9.28
E1	0.5	10	3	373.28 ± 12.11
E2	0.5	10	2	385.33 ± 6.06
E3	0.5	10	3	291.71 ± 3.45
F1	0.5	10	2	413.20 ± 25.40
F2	0.5	10	3	416.68 ± 29.65
F3	0.5	10	3	317.05 ± 0.73
G1	0.5	10	3	347.12 ± 23.59

The methane output from AD does provide an adequate amount of energy to return to the power grid, or recycle through the distillery, with a financial value of approximately $2.5 million for larger distilleries, or $250,000 to $500,000 for the smaller distilleries. While this information does not account for the capital expenses, it does provide an indication of the benefit potentially provided to the distillery. From a community standpoint, with the assumption of the annual household energy usage of

10,715 kWh, the energy from Distillery D could provide energy for approximately 3,200 households within the community.

While this study analyzed 14 stillage samples from seven distilleries, there are upwards of 80 more distilleries in the state with additional varying mash bills. The data in this study only scratched the surface of the possibilities for AD within the industry. Moving forward, the addition of some wheat base mash bills would be beneficial in drawing a conclusion about the impact of wheat in stillage on AD. Finding other rye varieties that fill in the gaps between the rye whiskies and low rye bourbons would allow for a more accurate regression to determine the linear relationship on rye (and corn) on the methane production.

Performance of an in-depth life cycle assessment (LCA) and techno-economic analysis (TEA) would better inform distilleries on the outcome of AD, and the potential return on investment for implementing AD into the distillery, based on capital and operating expenses, and determining if AD truly saves energy, or if the energy input into the reactors creates a net energy consumption near zero. Smaller, craft distilleries and larger, heritage distilleries have different energy requirements, and produce whiskey (and stillage) at differing volumes. With this, it is understood that AD might not benefit all distilleries, depending on size and location. Additional concepts to consider would be the benefit of including a digester central to a number of distilleries that would all send stillage there and the output would be shared.

Although analysis was performed on the digestate, more research could be performed in that area. This study reused the digestate as the inoculum in the BMPs that followed, but digestate has qualities allowing it to be used as a fertilizer. Distilleries such as Distillery G are hoping to produce their own crops. Using stillage in AD, then using the fertilizer for the grains creates a circular system within the distillery. The AD of stillage has potential in many ways, and some distilleries are recognizing that potential. In all, AD of stillage proves to be a viable option for distilleries by providing a potential energy source and although distillation parameters and mash bill impacted the BMP, the impact was not large enough to make AD a poor solution to the stillage issues in Kentucky.

APPENDICES

APPENDIX I. ABBREVIATIONS

Abbreviation	**Definition**
AD	Anaerobic digestion
AD fiber	Acid detergent fiber
ANOVA	Analysis of variance
APHA	American Public Health Association
BMP	Biomethane potential
BPC GE	Bioprocess Control Gas Endeavour
BSG	Brewers' spent grains
CH_4	Methane
CO_2	Carbon dioxide
COD	Chemical oxygen demand
.CSV	Comma separated values
DSG	Distillers' spent grains
EPA	Environmental Protection Agency
FM	Food to microbe ratio
H_2	Hydrogen
H_2SO_4	Sulfuric acid
KOH	Potassium hydroxide
LCA	Life-cycle assessment
LCFA	Long chain fatty acids

NaOH	Sodium hydroxide
ND fiber	Neutral detergent fiber
NIR	Near-infrared spectroscopy
OLR	Organic loading rate
RNG	Renewable natural gas
tCOD	Total chemical oxygen demand
TEA	Techno-economic analysis
TS	Total solids
VFA	Volatile fatty acids
VS	Volatile solids

APPENDIX II. TOTAL AND VOLATILE SOLIDS EQUATIONS

$$\% TS = \frac{C - A}{B - A} \times 100$$

$$\% VS = \frac{D - C}{B - A} \times 100$$

Where:

A = weight of dish (g)

B = weight of dish + sample (g)

C = weight of dish + sample after drying in an oven at 105°C (g)

D = weight of dish + sample after combustion in a furnace at 550°C (g)

APPENDIX III. LOADING RATE EQUATIONS

$$Substrate\ Loading = V \times OLR$$

$$Inoculum\ Loading = \frac{Substrate}{FM}$$

$$Substrate = \frac{Substrate\ Loading}{VS_{substrate}}$$

$$Inoculum = \frac{Inoculum\ Loading}{VS_{inoculum}}$$

$$Water = V - Substrate - Inoculum$$

Where:

V = working volume (L)

OLR = organic loading rate (g VS/L)

FM = food to microbe ratio (g VS/g VS)

Substrate Loading = amount of substrate loaded (g VS)

Inoculum Loading = amount of inoculum loaded (g VS)

Substrate = mass of substrate utilized (g)

Inoculum = mass of inoculum utilized (g)

APPENDIX IV. ALKALINITY EQUATIONS

$$Alkalinity\ (mg\frac{CaCO_3}{L}) = \frac{A \times N\ \times 50{,}000}{S}$$

Where:

A = mL standard acid used

N = normality of standard acid

S = mL of sample used

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