ABSTRACT

The Fate of Phosphate in the MixAlco Process and its Applicability to a Central Texas Watershed

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The MixAlco process is proposed as a means to reduce phosphorus concentrations in dairy cow manure in order to help improve water quality in the Lake Waco watershed. Numerous dairy farms and intensive agricultural practices are located in this Central Texas watershed, and dairy manure is a major source of nutrients, particularly phosphorus. Nutrients, such as nitrogen and phosphorus, are the main causes of eutrophication. The MixAlco process, which can use dairy manure as a source of biomass to produce a mixed alcohol fuel, may reduce phosphorous levels in manure wastes. The dairy manure filtrate was analyzed for soluble reactive phosphorous (SRP) before and after the first two steps of the MixAlco process. An average reduction of 86 percent was observed from beginning to end. A reduction in SRP may ease the impact dairy manure has on eutrophication in the Lake Waco watershed to help improve water quality.

The Fate of Phosphate in the MixAlco Process and its Applicability to a Central Texas Watershed

by

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A Thesis

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LIST OF ABBREVIATIONS

AC	Ash Content
ANOVA	Analysis of Variance
1B, 2B, 3B	Bromoform
cm	Centimeter
d	Day
DW	Dry Weight
g	Gram
GC	Gas Chromatogram
h	Hour
kg	Kilogram
L	Liter
mL	Milliliter
P	Phosphorus
1P, 2P, 3P	Pretreatment
1PB, 2PB, 3PB	Pretreatment and Bromoform
rpm	Revolution per Minute
SRP	Soluble Reactive Phosphorus
TP	Total Phosphorus
µg P/g DW	Microgram of Phosphorus per Gram of Dry Weight
µL	Microliter
VS	Volatile Solid

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DEDICATION

To Mom, Dad, Neal, Ryan, and Jonathan

CHAPTER ONE

Introduction

The majority of people in the United States are fortunate enough to have running water to meet their basic needs. Yet, the quantity of water is directly related to the quality of water. Higher levels of water contamination result in a decrease in useable water quantity (Carpenter et al. 1998, 560). As population and demand for water grow, particularly in drier areas of the country, maintaining availability of clean water is extremely important. The United States Environmental Protection Agency (USEPA) has worked diligently over the last three and a half decades to monitor water quality in order to comply with the Clean Water Act. However, despite the progress made by the U.S., many bodies of water remain polluted, and need continual protection.

Poor water quality can manifest in the form of excessive levels of nutrients and pesticides, turbidity, color, and low levels of dissolved oxygen, as well as a number of other biological and chemical parameters (McFarland et al. n.d., 1-2; Kalff 2002, 236-37; Martin and Cooke 1994, 25). For instance, inhabitants of Central Texas are familiar with odor and taste problems associated with their drinking water, which can be attributed to the process of eutrophication (McFarland, Kiesling, and Pearson 2001, 14). Eutrophication can be a natural phenomenon, whereby water bodies gradually change from a clear, well-oxygenated, oligotrophic state to a more turbid, oxygen-poor, eutrophic state (McFarland et al. n.d., 2; Kalff 2002, 21). Because of our large-scale intensive agricultural processes, the nutrient loading in water that causes eutrophication is

often dramatically elevated (McFarland and Hauck 1999), resulting in the degradation of water quality.

Dairy cow manure is a major source of nutrients, particularly phosphorus, potentially accelerating the eutrophication process in the Lake Waco watershed of Central Texas (McFarland and Hauck 1999). The MixAlco process is proposed as a means to reduce phosphorus concentrations in the watershed by consuming dairy cow manure on the farm through anaerobic digestion. In addition to consuming manure and the possibility of improving water quality, the MixAlco process produces marketable liquid fuels and chemicals, which may help alleviate the United States' dependence on nonrenewable resources such as petroleum. In order to determine the effectiveness of the MixAlco process for reduction of soluble reactive phosphorus (SRP), measurements were made throughout the pretreatment and fermentation steps of the MixAlco process. The centrifuged liquid, or the filtrate, of dairy cow manure was measured for SRP, which was at one point in contact with the solid portion of dairy manure. Throughout the paper the term "SRP concentrations in dairy manure" is used to refer to the concentration of orthophosphate in the reacting fluids of dairy manure.

Lake Waco Characteristics

Lake Waco is located in McLennan County, Texas, and contains a volume of 17,814 hectare-meters over a surface area of about 2,914 hectares (Sullivan 1995). Lake Waco receives roughly 74 percent of its total drainage area from the North Bosque River. The Middle Bosque River, Hog Creek, and the South Bosque River together contribute the remaining 26 percent (Adams, Easterling, and McFarland 2005, 1). The Lake Waco watershed includes these four tributaries, and encompasses a total area of 430,000

hectares (McFarland, Kiesling, and Pearson 2001, 11). The North Bosque River is the largest tributary in terms of both length and watershed area, totaling 97 miles (Texas State Historical Association 2001) and approximately 316,000 hectares, respectively (McFarland, Saleh, and Hauck 2000, 11).

Approximately 46,000 dairy cows are located within the North Bosque River watershed. Runoff and discharges from these dairy farms and feedlots help explain the impaired condition of the watershed (City of Waco 2003; McFarland and Hauck 1999). Between 1992 and 2005, Section 303(d) of Texas's impaired water body list listed segment 1226 and segment 1255 nine and seven times, respectively. (Texas Commission on Environmental Quality 1992, 1994, 1996, 1998, 1999, 2000, 2002, 2004). The water quality parameters of concern have changed from year to year and vary based upon the segment; however, excessive levels of bacteria and nutrients are consistently cited as reasons for the listing of these segments in the section 303(d) list since 1994 (Texas Commission on Environmental Quality 1994, 1996, 1998, 1999, 2000, 2002, 2004; Adams, Easterling, and McFarland 2005, 3). The Middle and South Bosque Rivers, comprising segment 1246, only appeared on the 303(d) list in 1992, citing excessive nutrients and fecal coliform bacteria as the major parameters of concern (Texas Commission on Environmental Quality 1992). While Lake Waco, segment 1255, has not yet been listed on the 303(d) list, it is only a matter of time before the reservoir is deemed impaired as a result of the already impaired tributaries and to the numerous dairies located upstream (City of Waco 2003; Texas Commission on Environmental Quality 1992, 1994, 1996, 1998, 1999, 2000, 2002, 2004; McFarland et al., n.d., 2). It is important to keep Lake Waco, and its tributaries, off the list of impaired water bodies

since they serve as the drinking water source for the City of Waco and other communities, providing roughly 150,000 people with water (Texas Natural Resource Conservation Committee 2001; Adams, Easterling, and McFarland 2005, 1; Scott and McFarland 2002, 9).

Eutrophication

Eutrophication is a phenomenon that may impair a body of water. It is a naturally occurring as well as a human induced process resulting in a nutrient rich lake or reservoir (Kalff 2002, 94; McFarland, Kiesling, and Pearson 2001, 14). Nutrients such as nitrogen and phosphorus are necessary for plant and animal growth; however, an overload can produce anoxic conditions and toxic algae blooms. These can affect the aquatic and/or the terrestrial ecosystem by decreasing species diversity, destroying habitats, and diminishing the food supply (McFarland et al. n.d., 2; McFarland, Kiesling, and Pearson 2001, 14). A related issue is the production of carcinogenic and mutagenic disinfectant by-products (DPBs) as a result of the chlorination step, which is needed to treat water supplies. DPBs may pose potential health risks on the human and animal populations, and "greatly enhance the production of" trihalomethanes (THMs), a group of DPBs (Martin and Cooke 1994, 24; Walker 1983, 39).

Eutrophication can have numerous negative affects on those relying on the water body, thus it is important to guard against eutrophication, particularly as a result of anthropogenic contributions. These anthropogenic contributions, also known as cultural eutrophication, appear to originate primarily from nonpoint sources and contribute to the poor water quality of the Lake Waco watershed (McFarland and Hauck 1999; McFarland et al. n.d., 2; McFarland, Kiesling, and Pearson 2001, 89-91).

Nonpoint sources are considered to be the most important contribution of nitrogen and phosphorus inputs to surface waters around the U.S. (Carpenter et al. 1998, 561). Agriculture and urban runoff are the major identifiable causes of nonpoint source pollution (Carpenter et al. 1998, 559; Sharpley et al. 1994, 437). While point sources of pollution are easier to control than nonpoint sources, point sources, such as animal feedlot and construction site runoff, may still have a significant impact in some areas (Carpenter et al. 1998, 560). In the Lake Waco watershed, "direct stormwater runoff from confinement areas and discharge of dairy process wastewater" in the upper North Bosque River were "not considered to be major sources of in-stream nutrients" due to the installation of "wastewater containment structures, such as lagoons," in the early 1900s (McFarland and Hauck 1999). Thus, the treatment lagoon themselves do not appear to be as significant a contributor to water quality problems in the Lake Waco watershed as nonpoint source agricultural operations (McFarland and Hauck 1999). Runoff from agricultural fields on which dairy manure has been spread is considered a nonpoint source, however, and such land application is the final destination for manure held in the holding ponds.

Agricultural operations are common in the North Bosque River, particularly dairying (McFarland and Hauck 1999; McFarland and Hauck 2001, 224). The upper North Bosque River, which refers to the headwaters of the North Bosque River, originates in "Erath County, the number one milk-producing county in Texas" (McFarland and Hauck 1999; Scott and McFarland 2002, 11). As the major tributary to Lake Waco, its water quality has an effect on the water quality in Lake Waco. For instance, there was an obvious gradient of chlorophyll-α concentrations within Lake

Waco, where the highest concentrations occurred "near the main inflow to Lake Waco, the North Bosque River, and decreasing[ed] with longitudinal distance and increasing depth towards the main body of the reservoir" (McFarland, Kiesling, and Pearson 2001, 89). Chlorophyll-α is an index used to help determine a lake's productivity, by indirectly measuring the amount of living algae present in the water (Kalff 2002, 328).

While chlorophyll-α concentrations help identify a lake's productivity, the Redfield ratio aids in the determination of the limiting nutrient (Kalff 2002, 329). When the supply of the limiting nutrient is constrained, algal growth is limited, and the control of this limiting nutrient may result in a decreased rate of eutrophication. Algae require numerous elements; however, in terms of growth limitation, nitrogen and phosphorus are the most important (Kalff 2002, 94, 247). Much focus has been placed on phosphorus in the Lake Waco watershed, particularly in the North Bosque River watershed, because of its high association with dairy waste application fields (McFarland and Hauck 1999; McFarland and Hauck 2001, 223; Hauck 2002, 3). In addition, phosphorus is considered to be the nutrient that, if reduced, will more effectively limit algal growth because it is easier to control than nitrogen (Texas Commission on Environmental Quality and Texas State Soils and Water Conservation Board 2003). Phosphorus is also most often the limiting nutrient in freshwater systems (Sharpley et al. 1994, 437), whereas nitrogen is most often the limiting nutrient in saltwater systems (Murphy 2002).

A study performed by McFarland, Kiesling, and Pearson (2001, 91) revealed that "over 90 percent of the nutrient-limitation bioassays showed a phosphorus limitation" in Lake Waco. However, the obvious phosphorus limitation was more obscure towards the end of summer and early fall, thus denoting a possible "colimitation of phosphorus and

nitrogen" (McFarland, Kiesling, and Pearson 2001, 91; Doyle, Scott, and Conry n.d.). Phosphorus limitation, as determined by the Redfield ratio, not only varied seasonally, but also varied spatially (McFarland, Kiesling, and Pearson 2001, 90-91). The magnitude of phosphorus limitation was highest at the southern arm of Lake Waco, and was less obvious towards the inflow of the North Bosque River (McFarland, Kiesling, and Pearson 2001, 89). As a result of the spatial and seasonal phosphorus limitation variations, nitrogen as a significant cause of eutrophication should not be ignored; however, the work of this project focuses on phosphorus due to its association with dairy waste application fields.

Phosphorus is also important because it has been correlated with water taste and odor problems and algal biomass (Smith et al. 2002, 322; Davies, Roxborough, and Mazumder 2004, 1900). For instance, a reduction in phosphorus concentrations may decrease taste and odor problems identified in a Kansas hypereutrophic reservoir (Smith et al. 2002). Further, Davies, Roxborough, and Mazumder (2004) found that phosphorus was the main trigger for algal biomass, producing odors in many lakes and reservoirs in British Columbia. The production of geosmin and 2-methylisoborneol (MIB) in reservoirs by algae are associated with taste and odor problems (Smith et al. 2002, 321; Kim et al. 1997, 29). Taste and odor problems in Lake Waco were noticed as early as 1967 (McFarland, Kiesling, and Pearson 2001, 13-14), and the inhabitants of Central Texas relying on Lake Waco as a drinking water source are still dealing with this issue.

Despite the apparent affects of algal biomass on the City of Waco's drinking source in terms of taste and odor problems, chlorophyll-α concentrations, an indirect measure of algal biomass, are not yet at a concerning level (McFarland, Kiesling, and

Pearson 2001, 13). Nutrient concentrations are also not yet at a concerning level in Lake Waco (McFarland, Kiesling, and Pearson 2001); however, specific segments within the watershed have appeared on the State of Texas Section 303(d) List for the past decade for reasons of high nutrient concentrations (Texas Commission on Environmental Quality 1992, 1994, 1996, 1998, 1999, 2000, 2002, 2004). Agricultural operations appear to be a major source of these high nutrient loadings (McFarland and Hauck 1999; McFarland, Kiesling, and Pearson 2001, 89-91; Texas Natural Resource Conservation Commission 1996; McFarland and Hauck 2001, 235), and "dairying is the dominant agricultural practice" in the North Bosque River watershed (McFarland and Hauck 1999). It is unlikely that the dairy farming industry in Central Texas will cease to exist despite increased costs associated with water quality compliance measures (Leatham et al. 1992, 2856), therefore the environmental realm, the dairy industry, and the agricultural industry must learn to collectively fight against water pollution.

Diary Farming and Central Texas

Dairy farming in Texas continues to be an important way of life for many residents (Odom 2004; Leatham et al. 1992, 2856). Dairy farming in Texas reached its all time high in 1949 with 1,283,000 dairy cows and 321,223 farms, before dairy cattle numbers dropped to 297,921 and 13,687 farms in 1974 (Odom 2004). While the amount of dairy cattle decreased, "the total amount of milk produced" remained constant, with only a small drop in production (Odom 2004). The amount of farms reached an even lower total of 5,899 in 1987; however, this drop was accompanied by a large increase in dairy cows totaling 356,538 (Odom 2004). By 2002, the number of dairy cows and dairy farms dropped to 309,058 and 2,080, respectively (United States Department of

Agriculture 2004a). Even though many farmers appeared to have relocated outside of Texas since 1949 (Odom 2004), Texas is still within the top 12 in the nation for the number of dairy cows and the number of dairy farms (United States Department of Agriculture 2004a).

The most popular dairy cow in Texas since the 1950s are Holsteins, because of their ability to produce large amounts of milk with little fat (Odom 2004). Before the 1950s, the Jersey was the favored breed because of its ability to produce "milk with a high butterfat content" (Odom 2004). The shift in the preferred cow was a result of the shift in the Texas milk market that occurred in the 1950s as a result of the specialization of dairy cows to produce only milk (Odom 2004).

Despite the market shift, the favored location of dairy farming has not changed. One decade ago, "95 percent of the dairy industry in Texas was located east of a line running from Wichita Falls to Brownwood to San Antonio to Corpus Christi" (Odom 2004). Erath, Hopkins, Comanche, and Johnson counties accounted for approximately "44 percent of the milk in Texas" (Odom 2004). Erath County is still considered a "leading milk-producing [county]" in Texas (Odom 2004); however, from 1997 to 2002 the number of cows decreased by 14,360, and the number of farms decreased by 110 (United States Department of Agriculture 2004b). Regardless of the drop in the number of dairy cows and dairy farms from 1997 to 2002, Erath County was number 1 and number 2 in the state of Texas as far as having the most cows and farms (United States Department of Agriculture 2004b). While there was not a change in the ranking, the number of dairy cows per farm has increased. If the majority of these dairy farms in

Erath County are located within the North Bosque River watershed, this could result in more nonpoint source pollution as a result of dairying.

The headwaters of the Upper North Bosque River are completely within Erath County, and the fact that it is "the number one milk-producing county in Texas" has significant impacts on the quality of the river as well as Lake Waco (McFarland and Hauck 1999). The North Bosque River watershed also covers most of Bosque, Somervell, and McLennan counties and parts of Coryell and Hamilton counties. At the time of a study performed by the Texas Natural Resource Conservation Commission (2001), approximately 104 dairy farms were located within the North Bosque River watershed, with the majority in Erath County.

The MixAlco Process

The MixAlco process may help reduce the impact that dairy farms have on the Lake Waco watershed by possibly reducing discharge and runoff from dairy farms. The process was developed by Mark Holtzapple of Texas A&M University to convert "any biodegradable material," such as manure, agricultural residues, sewage sludge, sorted municipal solids waste (MSW), industrial biosludge, or energy crops, into a mixed alcohol fuel or other product such as acetone or acetic acid (Holtzapple et al. 1999, 609). This process may be applied to Central Texas to improve water quality by converting dairy cow manure into a mixed alcohol fuel. The manure that would otherwise remain on the land and is subject to runoff into nearby bodies of water would be removed, and as a major source of nutrients, particularly phosphorus (McFarland and Hauck 1999), the rate of eutrophication may decrease and the associated affect may improve taste and odor conditions of the water. According to Holtzapple et al. (1999, 611-623), there are five

major steps of the MixAlco process, which include: lime pretreatment, fermentation, dewatering and drying, thermal conversion and hydrogenation (see figure 1.2). It is envisioned that only the first three steps of this process would be applied on-site at a dairy farm. The first two steps, pretreatment and fermentation, which are expected to reduce SRP levels in dairy manure, are the focus of this investigation.

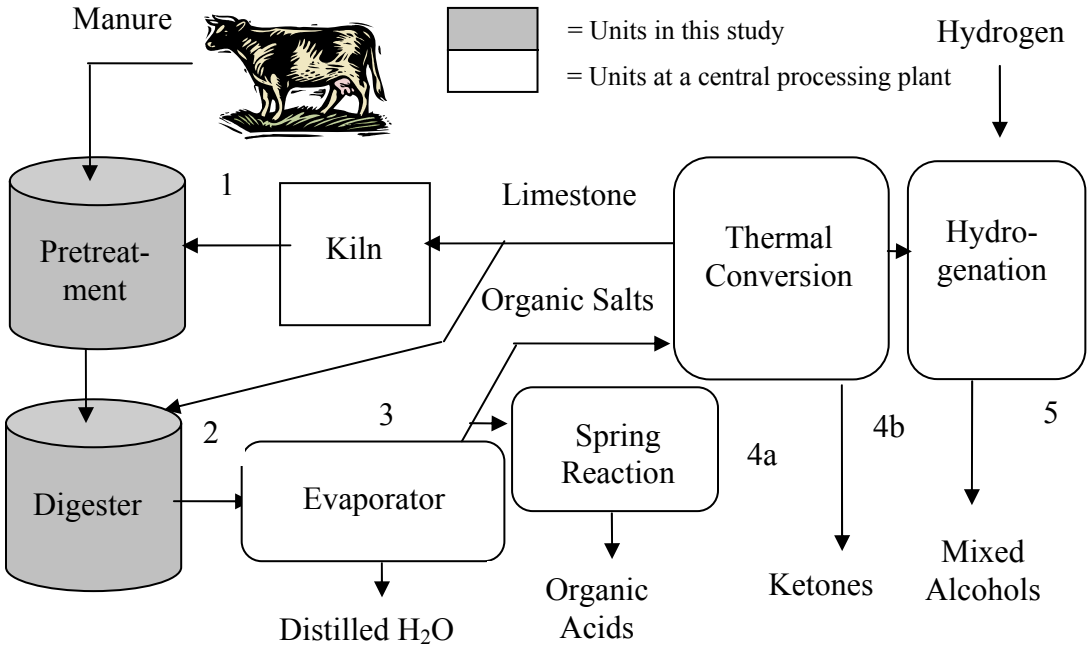


Figure 1.2. Unit operations of the MixAlco Process.

Lime Pretreatment

The purpose of the lime pretreatment step is to increase biomass digestibility, which is accomplished by the addition of lime (Holtzapple et al. 1999, 609; Chang, Burr, and Holtzapple 1997, 3; Kaar and Holtzapple 2000, 198; Gandi et al. 1997, 195; Chang et al. 2001b, 1). Lime has been shown to increase the digestibility of extracellular cellulases by 5.6 to 13.3 times, agricultural residues by about 2.0 times, and sorted municipal solid waste (MSW) by only 1.1 to 1.3 times (Holtzapple et al. 1999, 611). In

general, the more processed a waste stream, the less the pretreatment will improve digestibility. For example, in the case of MSW, many of its components, like copy paper, "have already been extensively alkaline treated in the paper-pulping process" (Holtzapple et al. 1999). Thus, the extent of biomass digestibility depends on its original state and source. Lime is predicted to moderately increase the digestibility of cow manure.

Fermentation

The purpose of the fermentation step is to first produce carboxylic acids, which are eventually converted into carboxylate salts once calcium carbonate is introduced to the fermentation process (Holtzapple et al. 1999, 609). Previous fermentation experiments have been carried out either in batch or continuous mode (Ross and Holtzapple 2001, 111; Aiello-Mazzarri, Agbogbo, and Holtzapple 2006, 47; Thanakoses, Mostafa, and Holtzapple 2003, 523; Chang et al. 2001a, 1327). Batch fermentations allow a relatively quick investigation of different process parameters, while a continuous culture, conducted with countercurrent solid and liquid flows, has been shown to optimize conversion productivity and yield (Holtzapple et al. 1999, 613). In this study, the fermentation inquiries will be carried out in batch mode to enable a more rapid evaluation of various process parameters.

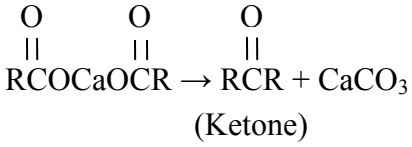
Dewatering and Drying

Holtzapple et al. (1999, 617-621) describe two steps in order to extract water from the carboxylate salts that are produced in the fermentation process. First, through a liquid extraction and separation process, an amine solution dewaters the carboxylate salts (Holtzapple et al. 1999, 617-618). Secondly, the carboxylate salts are precipitated with

the use of countercurrent heat exchangers and multi-effect evaporators (Holtzapple et al. 1999, 620-621). The precipitated salts are then further dried to complete this step of the process (Holtzapple et al. 1999, 621).

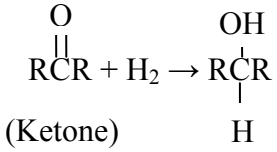
Thermal Conversion

Thermal conversion, as applied to the MixAlco method, is the process of decomposing carboxylate salts into ketones (Holtzapple et al. 1999, 621). Conversion into a ketone can be accomplished by heating the salts to extremely high temperatures (Holtzapple et al. 1999). A ketone, as seen in the following formula, is defined as an organic compound with two R groups attached to the carbon by a single bond, and oxygen forms a double bond with the carbon (Olmstead and Williams 1994, 390, 500).



Hydrogenation

Hydrogenation is the final step in converting biomass into a mixed alcohol fuel (Holtzapple et al. 1999, 622-623). The R groups in the ketone "can be almost any organic fragment as long as the atom bonded directly to the carbonyl (the carbon-oxygen double bond) is a carbon," (Olmstead and Williams 1994, 390, 500). As indicated by the following equation, the process of hydrogenation converts the ketone into an alcohol by



the process of hydrogenation converts the ketone into an alcohol by adding a hydrogen bond to the oxygen and carbon molecules, (Olmstead and Williams 1994), The double bond between the oxygen and carbon is broken, and a hydrogen atom takes its place (Olmstead and Williams 1994).

Renewable Energy

In addition to possibly improving water quality, the MixAlco process is advantageous in that it produces renewable energy. Energy is extremely important in today's society and is defined as the "capacity for vigorous action; inherent power; potential forces" (Hinrichs and Kleinbach 2002, 2). It "is one of the major building blocks of modern society" because without energy, many of the goods and services we value today would not be possible (Hinrichs and Kleinbach 2002, 1). Energy is produced by a number of commonly known sources such as hydroelectric power, nuclear electric power, oil, coal, and natural gas. In the United States, coal was the primary source of energy in the early to mid 20th Century until oil became the favored source in the last half of the 20th Century (Hinrichs and Kleinbach 2002, 11). However, oil is becoming a more and more unfavorable energy source, particularly in the U.S., due to public concerns about global warming, and its unstable market supply (Hinrichs and Kleinbach 2002, 284, 21-24).

Global warming is the result of greenhouse gases that are released into the air and increase the earth's temperature over time. Greenhouse gases, in particular carbon dioxide, are accumulating in the atmosphere mainly from the combustion of fossil fuels such as oil. The greenhouse effect is a phenomenon that results when greenhouse gases

"absorb certain wavelengths of infrared radiation emitted from the earth that would otherwise radiate out to space" (Hinrichs and Kleinbach 2002, 285). The trapped radiation raises atmospheric temperatures. In fact, according to climate models, the Earth's mean surface temperature is projected to rise one to three and a half degrees Celsius (Miller 2000, 503). A warmer world would change all aspects of life from food production and water supplies to biodiversity (Miller 2000). The effects are so dramatic that efforts to reduce the impact of global warming must be initiated now. Reduction in the use of fossil fuels is the first step toward that end.

In addition to the need to reduce the use of fossil fuels because of concerns for global warming, the unstable oil market supply has tended to put at risk the future of oil as a reliable source of energy. The decrease in world oil reserves and global political issues has contributed to its instability (Hinrichs and Kleinbach 2002, 21-24). As of 1998, the U.S. only had approximately 2.2 billion barrels of oil reserves as compared to the world reserve of 1.02 trillion barrels (Hinrichs and Kleinbach 2002, 205). At this time, the U.S. reserves represented roughly 8 years of sustained energy use (Hinrichs and Kleinbach 2002). It is no surprise then that the U.S. is projected to import up to 70 percent of its oil supply by 2010, a possible 15 percent jump from 1997 imports (Miller 2000, 372). The current war with Iraq puts imported oil from the Persian Gulf area at risk, particularly when the Middle East contains approximately 41 percent of the world oil reserves (Hinrichs and Kleinbach 2002, 207).

The combined threat of global warming and an unstable oil market supply has made the need for alternate energy sources more and more vital to the interests of the U.S. Perhaps the recent rise in oil prices will also make alternate energy sources more

appealing. While sources such as wind and solar energy are being explored, the most likely renewable resource to be used for displacing significant amounts of oil is biomass (Ristinen and Kraushaar 1999, 151).

Biomass energy is "energy derived from living matter such as" corn, wheat, trees, and water plants; "it is also agricultural and forestry wastes" such as crop residues and manure, and municipal solid wastes (Hinrichs and Kleinbach 2002, 540). Biomass can be further converted into fuels such as solid biomass fuels, liquid fuels, and gaseous fuels (Hinrichs and Kleinbach 2002). The MixAlco process offers an alternative to convert biomass into a mixed alcohol fuel. Manure as the source of biomass may improve water quality, while at the same time offering an alternate source of energy.

Phosphorus in Dairy Cow Manure

As a renewable energy source, manure looks promising, but to assess cow manure as a pollutant, phosphorus requirements and forms must be evaluated. The two major routes of phosphorus excretion in dairy cows are manure and milk, whereas very little is excreted in urine (Morse et al. 1992, 3048). The amount excreted in manure is directly related to the amount consumed (Morse et al. 1992, 3047), and phosphorus concentrations in milk are "associated with the quantity of milk produced and the content of milk constituents" (Morse et al. 1992, 3039). The digestibility or availability of phosphorus in manure is then dependent on the amount required for maintenance and milk production (Satter and Wu 1999, table 1).

Satter and Wu (1999) compared dairy cow phosphorus requirements of five different countries: United States, Netherlands, Great Britain, France, and Germany. France allots the largest amount of phosphorus for maintenance (0.062 g/kg of BW)

followed by the Netherlands (0.042 g/kg of BW), Germany (0.040 g/kg of BW), the U.S. (0.0286 g/kg of BW), and Great Britain (0.0207 g/kg of BW) (Satter and Wu 1999, table 1). Phosphorus requirements for milk production range from 1.25 to 1.98 g/kg fat-corrected milk (FCM), with France requiring the least and the U.S. requiring the most (Satter and Wu 1999). The percent availability is then calculated by multiplying the maintenance and milk production phosphorus requirements (Satter and Wu 1999). The percent availability for the U.S is 50 percent as compared to 58 percent for Great Britain, 60 percent for Germany and the Netherlands, and 70 percent for France (Satter and Wu 1999). Based on many studies directly measuring phosphorus availability, Satter and Wu (1999, 73) conclude that 70 percent availability is the most accurate. Cows will absorb the necessary amounts of phosphorus, while the remainder is excreted (National Research Council 1989).

Phosphorus in dairy cow manure exists in the inorganic and organic form (Barnett 1994, 140). Inorganic phosphorus, mostly consisting of orthophosphate (Barnett 1994, 139), also referred to as soluble phosphorus, is biologically available for aquatic plants such as algae (McFarland and Hauck 1999; Dou et al. 2000, 508), thus aiding in their existence and growth. Organic phosphorus sources are "bound to plant or animal tissue" (Murphy 2002), and can be excreted as residual phosphorus, acid-soluble organic phosphorus, and phospholipids (Barnett 1994, 140). Fecal phosphorus may be classified into three different groups: unavailable phosphorus, inevitable phosphorus loss, and regulated phosphorus (Spiekers et al. 1993). The unavailable phosphorus is not absorbed endogenously, and is excreted as mostly "organic and water-insoluble plant cell wall residues" (Dou et al. 2002, 2063). The inevitable loss of phosphorus consists of

relatively insoluble organic sources such as microbial residues and sloughed gut tissue (Wu, Satter, and Sojo 2000, 1037). Digestive excretions represent the smaller portion of the inevitable loss of phosphorus, but are water-soluble (Dou et al. 2002). The regulated component of fecal phosphorus is dependent on dietary phosphorus (Spikers et al. 2000). As dietary phosphorus increases, excretion of the inorganic soluble fraction of phosphorus in manure should also increase. As a regulated source of phosphorus, the excreted inorganic fraction may be controlled by reducing the amount of phosphorus found in animal feed (Dou et al. 2002). This may help alleviate the impact of nutrient enrichment on nearby bodies of water as a result of manure runoff.

In order to reduce excreted phosphorus, many researchers advocate reducing dietary phosphorus (Dou et al. 2002, 2058; Ebeling et al. 2002, 290; Satter and Wu 1999,75-76; Morse et al. 1992, 3048; Dou et al. 2003, 3794). The National Research Council suggests a dairy diet of 0.34 to 0.41 percent phosphorus on a dry basis, yet many dairy farmers exceed these recommendations feeding their cows a dietary phosphorus average of 0.48 percent (Satter and Wu 1999). Dou et al. (2003, 3787) also noted that dairy farms in New York, Pennsylvania, Delaware, Maryland, and Virginia were also exceeding phosphorus feed rations by 34 percent equaling 0.44 percent dietary phosphorus. A reduction in dietary phosphorus by 0.1 percent, 0.48 to 0.38 percent, would result in a 25 to 30 percent reduction in fecal phosphorus (Satter and Wu 1999, 79). Also, fecal phosphorus increases by 0.008 percent for every 0.01 percent increase in dietary phosphorus (Morse et al. 1992). Reducing dietary phosphorus may be a logical and efficient way to improve water quality; however, because phosphorus levels are associated with milk yields and reproductive performance, many dairy farmers will not

chance the survival of their business to regard for the environment (Dou et al. 2003, 3794). Thus, the MixAlco process is a plausible solution to potentially aid in the reduction of excessive phosphorus levels in the Lake Waco watershed.

Route of Phosphorus in the MixAlco Process

The route of phosphorus through the MixAlco process is assessed as an alternative way to reduce phosphorus loadings in the Lake Waco watershed. However, the fate of phosphorus in the MixAlco process is not entirely clear, thus some guesswork is involved in order to determine its form. Before dairy cattle manure is pretreated with lime, $Ca(OH)_2$, the phosphates are likely to remain in solution, but with the addition of lime, calcium is added to the process, making Ca^{2+} present. Phosphorus is then able to precipitate out as hydroxyapatite ($Ca_5OH(PO_4)_3$) (Koschel 1997). Hydroxyapatite may be produced according to the following formula (van Loon and Duffy 2000, 360):

$$5Ca(OH)_2\ (aq) + 3HPO_4^{2-}\ (aq) \rightarrow Ca_5OH(PO_4)_3\ (s) + 6OH^-\ (aq) + 3H_2O$$

Lime increases the pH of the manure, for example, lime pretreatment increased the pH of switchgrass to a high of 11.5 (Chang, Burr, and Holtzapple 1997, 7). Calcium phosphates, like hydroxyapatite, are more insoluble at a higher pH, or a more alkaline solution (van Loon and Duffy 2000).

There is much evidence that lime will precipitate soluble forms of phosphorus into insoluble forms. For instance, in wastewater treatment plants, lime is able to precipitate 85 to 90 percent of the inorganic orthophosphates (Koutsoukos 2004). In addition, Prepas et al. (2001, 1057) showed that "lime applications can control P [phosphorus] concentrations…in eutrophic hardwater lakes." Lind (2002) also noted that to control phosphorus, the addition of calcium, such as lime, quicklime, calcium oxide,

calcium chloride, calcium sulfate, produced an insoluble calcium salt. The use of lime in the pretreatment step of the MixAlco process may reduce soluble phosphorus concentrations found in the filtrate associated with dairy manure, thus possibly improving the water quality of Central Texas.

Conclusion

To conclude, the MixAlco process would appear to be beneficial to Central Texas. It may not only improve water quality, but would also offer an alternative source of renewable energy. The problem with manure discharge and runoff from dairy farms will not subside unless action to reduce the impact continues and alternatives are explored. The MixAlco process is one alternative that may have promise.

Project Objectives

This project focused on the measurement of SRP concentrations in dairy manure as it was applied to the MixAlco process. Variables such as aeration, lime loading, and temperature and time were tested in order to determine which more effectively reduced SRP concentration in lime pretreated dairy manure. SRP concentrations were also measured in fermented dairy manure to determine which variables more effectively reduced SRP concentrations. Fermentation variables included temperature, pretreatment, and bromoform. The fermentation variables were also evaluated to determine the effects on acid and gas production, and pH. The dry weight, ash content, and volatile solids were measured throughout the process to determine the effects of pretreatment and fermentation. In short, the fate of phosphate was the main objective of this project, while

variables relevant to the performance and economic potential of the process were also evaluated.

CHAPTER TWO

Materials and Methods

Construction of Equipment

Roller Bottles

The roller bottles that housed the pretreated manure throughout the fermentation process were first constructed (see figure 2.1). The roller bottles were built based upon suggestions made by Ross and Holtzapple (2001, 114-115), but were adapted to fit the needs of this experiment. These bottles consisted of 500 milliliters (mL) nalgene bottles fitted with a 7 1/2 size rubber stopper. Two 1/4 inch holes and one 3/8 inch hole were drilled into the stopper, and 2 pieces of stainless steel tubing and one glass vial were fit into these holes to plug them. The stainless steel tubing was cut into approximately 10 inch pieces in length and each were bent at one end and welded and kinked at the other. The welded and kinked end fit inside the bottle, whereas the cane-like end stuck out of the bottle. The welded end was wrapped in shrink-wrap to avoid rusting. The bottom of the glass vial was filed off and the small opening in the lid was sealed with a septum, allowing for gas sampling. The inside of the nalgene bottle lid was cut out, leaving an opening with a diameter of approximately 1 1/4 inches. The lid was then used to seal the stopper onto the bottle, allowing for the stainless steel and glass vial to fit through the hole cut in the lid. It was necessary to shave down parts of the lid to allow a better fit over the stopper.

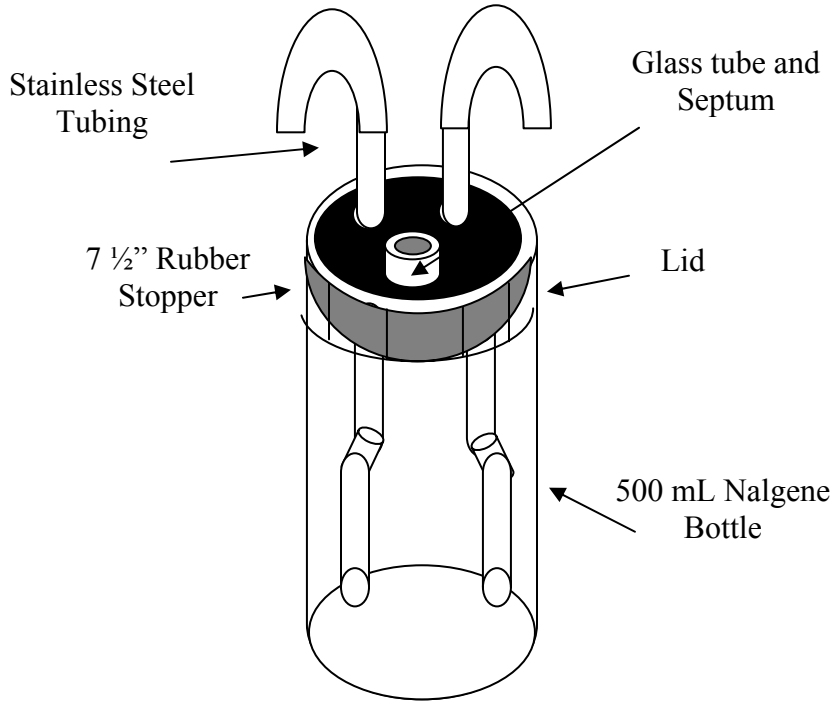


Figure 2.1. Schematic drawing of the fermentation roller bottle apparatus.

Gas Collecting Apparatus

The gas collecting apparatus (see figure 2.2) was also based on previous experiments conducted by Thanakoses, Mostafa, and Holtzapple (2003, 531), but were adapted to fit the needs of this experiment. The apparatus consisted of 2 pieces of lexane tubing cut at 24 and 30 inches. Both had a thickness of 1/8 inch and an inside diameter of 1 3/4 inch. Two pieces of lexane having the same dimensions were cut to fit inside the tubes at only one end. The end was then sealed with methylene chloride (CH_3Cl). A hole was drilled in the sealed end and threaded with a 1/4 inch tap. A male National Pipe Thread (NPT)/hose barb fitting was then inserted into the tapped hole. Rubber tubing was connected from the fitting to a vacuum source. The end of a syringe was cut off to connect to the other end of the rubber tubing allowing for the measurement of gas samples. Both lexane tubes were held upright with their open ends immersed in a

solution of calcium chloride ($CaCl_2$) and water (Thanakoses, Mostafa, and Holtzapple 2003). Measuring tape, calibrated to measure the gas displacement was permanently attached to the lexane tubing, which thus enabled measurement of collected gas volumes.

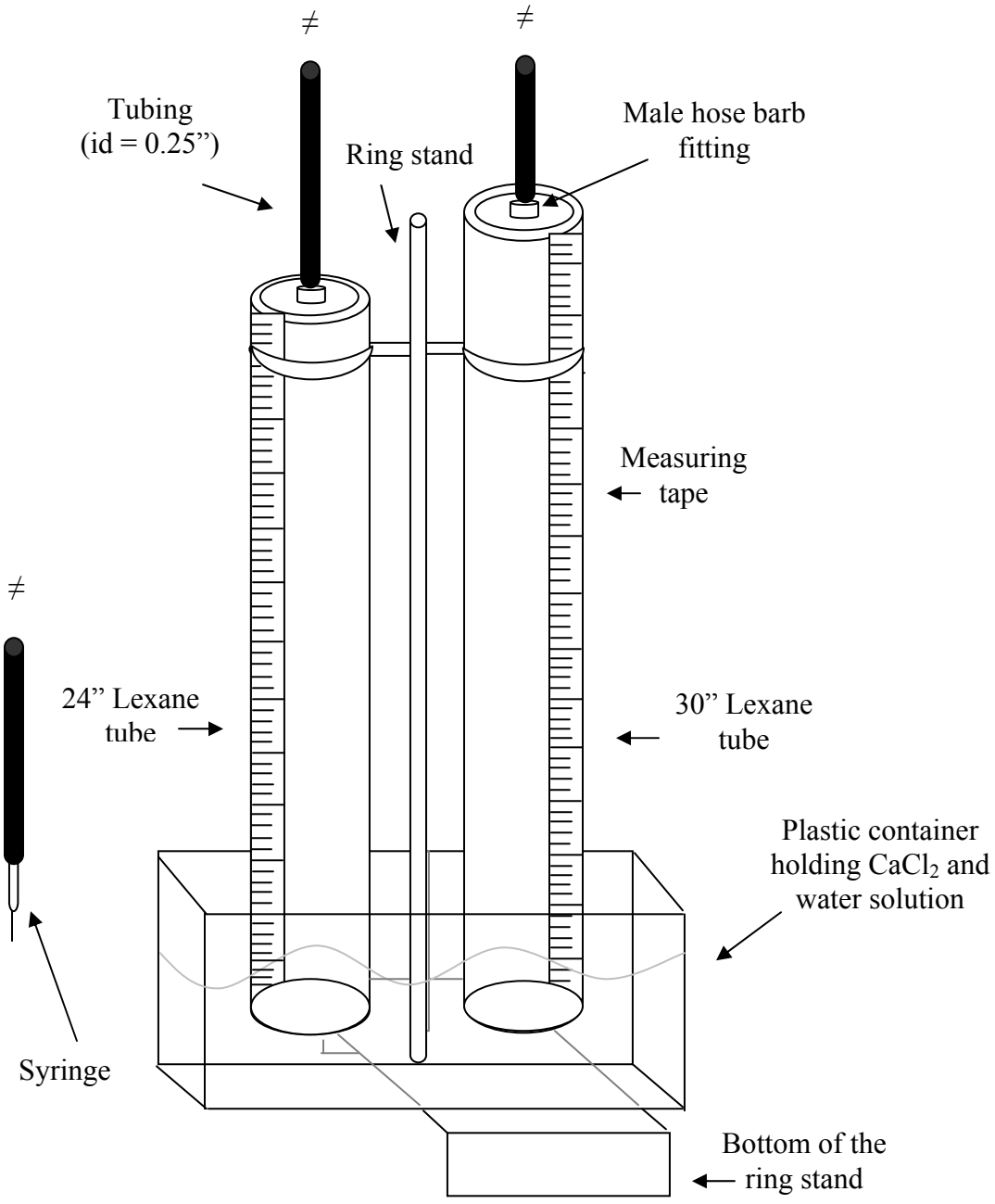


Figure 2.2. Schematic drawing of the gas collection apparatus.

Procedures

Sample Collection

Dairy cow manure was collected from a dairy farm located in Waco, Texas. Batch 1 was collected on March 21, 2005, batch 2 was collected on May 25, 2005, and batch 3 was collected on June 9, 2005. The manure was collected directly from a holding lagoon and placed in a 13.6 kilogram (kg) bucket. After the first batch of manure was collected, the dairy cattle farmer had the left side of the lagoon closed off, thus future batches were collected from the right side (see figure 2.3). The bucket of manure was then placed in the lab's refrigerator to be stored until needed.

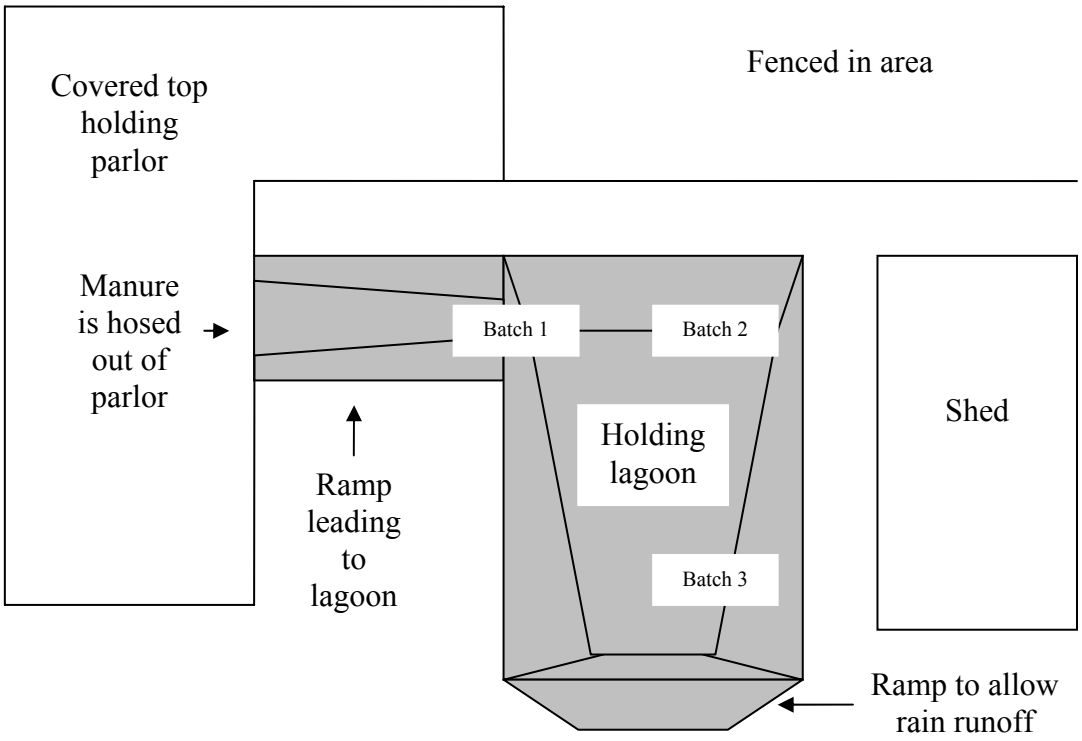


Figure 2.3. Schematic drawing of the holding lagoon.

Lime Pretreatment

Before the cattle manure was pretreated with lime, the dry weight was decreased from 17.80 (batch 1), 30.51 (batch 2), and 18.15 percent (batch 3) solids to approximately 10 percent (Kaar and Holtzapple 2000, 192). The percent moisture content was determined to ensure that it was around 90 percent. Once the moisture content was adjusted, the filtrate from the diluted manure was analyzed for SRP and TP using the ascorbic acid (SRP and TP) and persulfate digestion method (TP), and the moisture, ash, and volatile solid content were determined. Throughout the paper the term "SRP concentrations in dairy manure" is used to refer to the concentration of orthophosphate in the reacting fluids of dairy manure.

Lime ($Ca(OH)_2$) was then added to the diluted manure at a proportion of 0.1 gram (g) for every 1 g dry weight (DW) of manure (Holtzapple et al. 1999, 611; Chang, Burr, and Holtzapple 1997, 16). The 12 L cooking pot containing the lime and manure mixture was placed on a hot plate and cooked for 2 hours (h) at 100ºC (see figure 2.4) (Holtzapple et al. 1999; Chang, Burr, and Holtzapple 1997). The mixture was too thick for the magnetic piece that is designed to spin to agitate the contents. So, instead the mixture was thoroughly stirred occasionally throughout the 2 hours by hand with a spoon. At the end of the 2 hours the pH of the lime pretreated manure was adjusted with the bubbling of carbon dioxide (CO_2) to reach a near neutral pH (Ross and Holtzapple 2001, 117). The manure was then analyzed for SRP and TP, and the moisture, ash, and volatile solid content were determined.

In addition to pretreating dairy cow manure with 0.1 g of lime for every 1 g DW of manure (10 percent lime), as was seen in batches 1 and 2, dairy cow manure was also

pretreated with 0.05 g of lime for every 1 g DW of manure (5 percent lime) (batch 3). A second set of parameters was tested in accordance with the varying lime loadings in batch 3 (see table 2.1). An equal amount of manure was added to 12 500 mL Nalgene bottles. Six of these bottles contained 0.1 g of lime for every 1 g DW of manure, and the other 6 contained 0.05 g DW of lime for every 1 g of manure. Six of the 12 bottles, 3 containing 0.1 g of lime and 3 containing 0.05 g of lime for every 1 g DW of manure, were pretreated for 2 hours (h) at 100°C, and the remaining 6 were pretreated for 7 days (d) at 40°C. Of the 6 bottles pretreated at 100°C and the 6 pretreated at 40°C, air was bubbled through a total of 8, or 4 at each temperature.

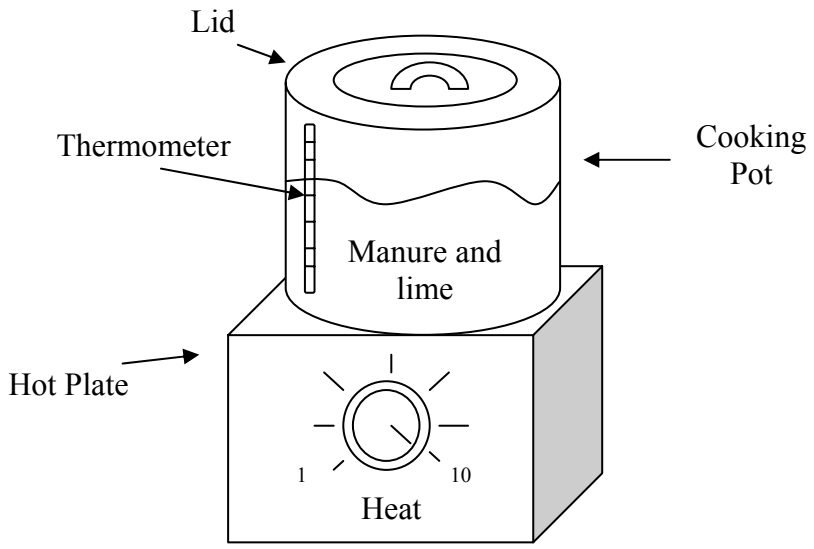


Figure 2.4. Schematic drawing of the pretreatment of dairy cow manure for 2h at 100°C (batches 1 and 2).

In order to remain consistent with the pretreatment methods performed in batches 1 and 2, the 6 bottles pretreated for 2 h at 100°C were placed in the 12 L cooking pot (see figure 2.5). The cooking pot contained a sufficient amount of water to cover the manure and lime mixture housed in the bottles. The 6 bottles were taped to an additional bottle

full of only water so that the bottles would not float. The 6 bottles pretreated for 7 d at 40°C were placed in an incubator (see figure 2.6). A system of hoses, with an inside diameter (id) of 0.125" and 0.25", and connectors, both Y and reducing connectors, were attached from an air pump (Aircadet Vacuum/Pressure Station, Barnant Co., Model No. 400-3901) to 4 of the 6 bottles to allow an equal flow of air. The 0.125" hose fit inside the 11/64 inch hole in the lid to rest in the sample in order to bubble air through the manure and lime. Air was able to escape the bottle through a 5/64 inch hole in the lid. Only one air pump was available, thus pretreatment at 100°C and 40°C occurred at different times. Initially, the air pump was placed inside the incubator; however, on the first day it overheated and was thus moved outside the incubator. Once the 2 h or 7 d time period expired, the filtrate from the pretreated dairy cow manure was analyzed for TP and SRP and the moisture, ash, and volatile solid content were determined.

Table 2.1. Pretreatment conditions of batches 1, 2, and 3.

Batches 1 and 2				
12 L Pot	Lime (g/g DW of manure)	Time	Temperature (ºC)	Air (Yes/No)
1	0.10	2	100	N
Batch 3				
Bottle	Lime (g)	Time (hours/days)	Temperature (ºC)	Air (Yes/No)
1	0.10	2 h	100	Y
2	0.10	2 h	100	Y
3	0.10	2 h	100	N
4	0.05	2 h	100	Y
5	0.05	2 h	100	Y
6	0.05	2 h	100	N
7	0.10	7 d	40	Y
8	0.10	7 d	40	Y
9	0.10	7 d	40	N
10	0.05	7 d	40	Y
11	0.05	7 d	40	Y
12	0.05	7 d	40	N

Batch Fermentation

The fermentation process of batch 1 began on April 5, 2005 and ended on May 5, 2005 while the process of batch 2 began on June 3, 2005 and ended on July 3, 2005. See table 2.2 for the fermentation conditions under which batches 1 and 2 operated. Batch 3 ended with pretreatment, thus fermentation did not occur.

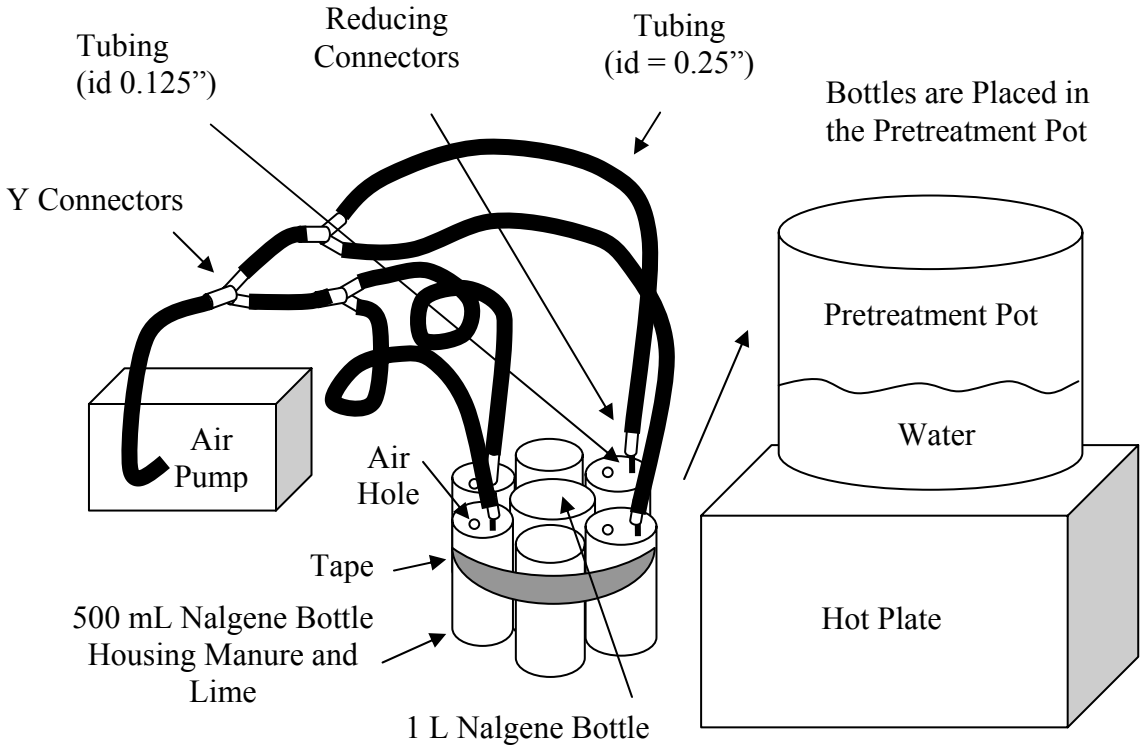


Figure 2.5. Schematic drawing of the pretreatment of dairy cow manure for 2h at 100°C (batch 3).

Once the manure was pretreated with lime, approximately 225 mL of the pretreated manure was transferred to each of the twelve roller bottles in batch 1 and to only 6 of the roller bottles in batch 2. Nonpretreated manure (with approximately 90% moisture content) was transferred to the remaining 6 bottles in batch 2. The roller bottles housing the manure were then separated into 2 incubators operating at 40ºC and 60ºC

(batch 1) and 40°C (batch 2) (Ross and Holtzapple 2001, 117; Thanakoses, Mostafa, and Holtzapple 2003, 528; Aiello-Mazzarri, Agbogbo, and Holtzapple 2006, 50), both rotating the bottles at 1 revolution per minute (rpm) (Thanakoses, Mostafa, and Holtzapple 2003) for a 4 week period.

Throughout this 4-week period, gas production and the pH of the fermenting bottles were measured daily in addition to the collection of a gas and liquid sample. The gas and liquid samples were analyzed with a Gas Chromatograph (GC) (Shimadzu model GC-2010). At the end of fermentation, the filtrate from the fermented manure in both batches was analyzed for SRP and TP, and the moisture, ash, and volatile solid content were determined.

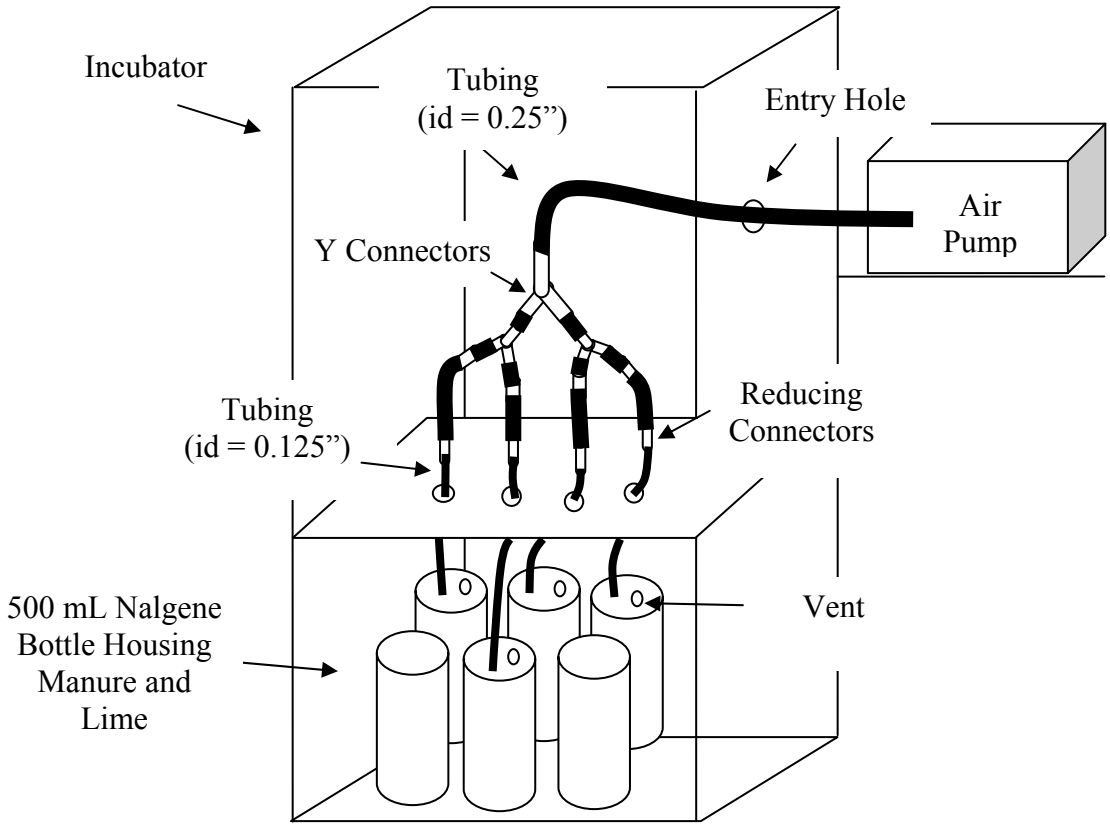


Figure 2.6. Schematic drawing of the pretreatment of dairy cow manure for 7d at 40°C (batch 3).

Table 2.2. Fermentation procedures for batches 1 and 2.

Batch 1					
Bottle	Organic Sediment (g)	Time (days)	Temperature (ºC)	Pretreated (Yes/No)	Bromoform (Yes/No)
1a	~0.7	30	60	Y	Y
2a	~0.7	30	60	Y	Y
3a	~0.7	30	60	Y	Y
4a	~0.7	30	60	Y	Y
5a	~0.7	30	60	Y	Y
6a	~0.7	30	60	Y	Y
1b	~0.7	30	40	Y	Y
1b	~0.7	30	40	Y	Y
3b	~0.7	30	40	Y	Y
4b	~0.7	30	40	Y	Y
5b	~0.7	30	40	Y	Y
6b	~0.7	30	40	Y	Y
Batch 2					
Bottle	Organic Sediment (g)	Time (days)	Temperature (ºC)	Pretreated (Yes/No)	Bromoform (Yes/No)
1	~0.7	30	40	N	N
2	~0.7	30	40	N	N
3	~0.7	30	40	N	N
1B	~0.7	30	40	N	Y
2B	~0.7	30	40	N	Y
3B	~0.7	30	40	N	Y
1P	~0.7	30	40	Y	N
2P	~0.7	30	40	Y	N
3P	~0.7	30	40	Y	N
1PB	~0.7	30	40	Y	Y
2PB	~0.7	30	40	Y	Y
3PB	~0.7	30	40	Y	Y

Inoculation

In order to speed up the conversion of biomass into carboxylic acids, approximately 0.7 g of organic sediment from a saline environment (salt marsh) was added to each bottle in both batches 1 and 2 before the start of the experiment (Thanakoses, Mostafa, and Holtzapple 2003, 527). This was done with the intent of

establishing a halophillic culture capable of thriving amid high concentrations of salts resulting from neutralization of the acid fermentation products.

Methane Inhibitor

A bromoform ($CHBr_3$) solution (Holtzapple et al. 1999, 613) was added to the acidogenic fermentations in order to inhibit the formation of methane (CH_4). The solution consisted of 20 g of the inhibitor (EM Science, Lot No. 41010113) per every L of ethanol (Thanakoses, Mostafa, and Holtzapple 2003, 527). Additions of the bromoform solution were made at the beginning of fermentation (75 microliters (μL)) and at intermediate points during the experiment (50 to 1000 μL and/or 50 to 200 μL of pure bromoform), immediately following the removal of the liquid sample, if methane production was detected. The quantity of bromoform added to the bottles depended upon CH_4 concentrations and the repeated appearances of CH_4. Pure bromoform was eventually added to the bottles in batch 2 due to the apparent ineffectiveness of the bromoform/ethanol solution. If a methanogenic state was desired for the fermentation, no addition of bromoform was made. The solution was stored in a tinted bottle, and immediately after each use, the cap was replaced due to "light and air sensitivity" (Thanakoses ,Mostafa, and Holtzapple 2003, 527-528).

Gas Production/Sampling

As the fermentation progressed, pressure built up in the bottles as a result of the formation of gases. So that the bottles would not explode, gases were released daily into the gas collecting apparatus to determine the volume that was produced. Before the gases were released, a 1 mL sample of gas was removed by use of a syringe, and that gas

sample was then analyzed for CH_4 and CO_2 with the GC. Next, in order to measure the volume of gases present in the bottle, the vacuum was first turned on to fill up the gas collection tubes with the $CaCl_2$ solution. The gas was released into the tubes by inserting the needle into the bottle via the septum, and the meter tape was used to determine the point at which the solution was displaced to denote the gas volume. At times, the needle would clog due to the occasional liquid release, thus the needle was replaced periodically. The septum was changed weekly to reduce the chance of any leakage through the small insert holes as a result of the needle.

Nitrogen Purge

To prevent oxygen contamination, the bottles were continuously purged with nitrogen (Airgas, Inc., zero grade) while they were open to the atmosphere (Ross and Holtzapple 2001, 119).

pH

Once the gas production of each bottle was measured, the lid and stopper were removed. The bottle was then immediately purged with nitrogen and a pH measurement was made once it was agitated. If the measurement indicated a low pH (<6.0), horticultural hydrated limestone (Hi-Yield) was added to the fermentor bottle before resuming the fermentation (Thanakoses, Mostafa, and Holtzapple 2003, 528). The amount of hydrated limestone ($CaCO_3$) added was based on the level of the pH, and the total amount that was added thus far.

Liquid Sampling

After the pH measurement was made, the bottles were agitated once more in an attempt to homogenize the contents, and approximately 3 mL were extracted from each bottle (Ross and Holtzapple 2001, 118). The liquid sample was then placed in a 3 mL centrifuge tube, and was centrifuged for 10 minutes at a speed of 10,000 rpm to separate the solids from the liquid. The liquid was then pipetted to another centrifuge tube and centrifuged again for 10 minutes at 10,000 rpm. Multiple centrifuging occurred in order to avoid contamination of the GC column due to the presence of solids in the sample. The centrifuged samples were then stored in the freezer until analysis could occur.

Analyses

Moisture Content

In order to determine the moisture content of the sample, numerous subsamples were dried in an oven, (Sheldon Manufacturing, INC. model number 1320), set at 60°C for 48 hours. The percent moisture and dry weight were determined by the following formulas (van Walsum 1999):

Percent moisture = 100 X (1-(mass of dry sample / mass of wet sample))

Dry Weight = 100 X (mass of dry sample / mass of wet sample)

The moisture content and dry weight of the manure were determined before and after the applied steps of the MixAlco process.

Ash Content

Dry solids from the manure were placed in crucibles and ashed in a furnace at 650ºC for 30 minutes (Castleberry 2001). The percent ash content and volatile solid content were determined by the following formulas:

Percent ash content = 100 X (mass of ashes / mass of dry sample)

Percent volatile solids = 100 X (1- (mass of ashes / mass of dry sample))

The ash and volatile solid content of the manure were determined before and after the applied steps of the MixAlco process.

Soluble Reactive Phosphorus and Total Phosphorus

The samples were prepared and analyzed for soluble reactive phosphorus (SRP) and total phosphorus (TP) by the ascorbic acid method (SRP and TP) and persulfate digestion method (TP). Refer to figures 2.7 and 2.8 for a diagram of sample preparation and SRP and TP analysis. The manure filtrate was analyzed for SRP and TP before and after the applied steps of the MixAlco process.

Ascorbic Acid Method

Sample Preparation. Eight samples were used for measuring SRP in diluted and pretreated dairy cow manure in batches 1 and 2. Twelve samples were used, 2 subsamples from each fermentor bottle, for the fermentations. Twelve samples, 2 subsamples from each bottle, of batch 3 were used for SRP and TP analysis of pretreated manure. Thus, the following steps were performed simultaneously for the 8 or 12 manure samples.

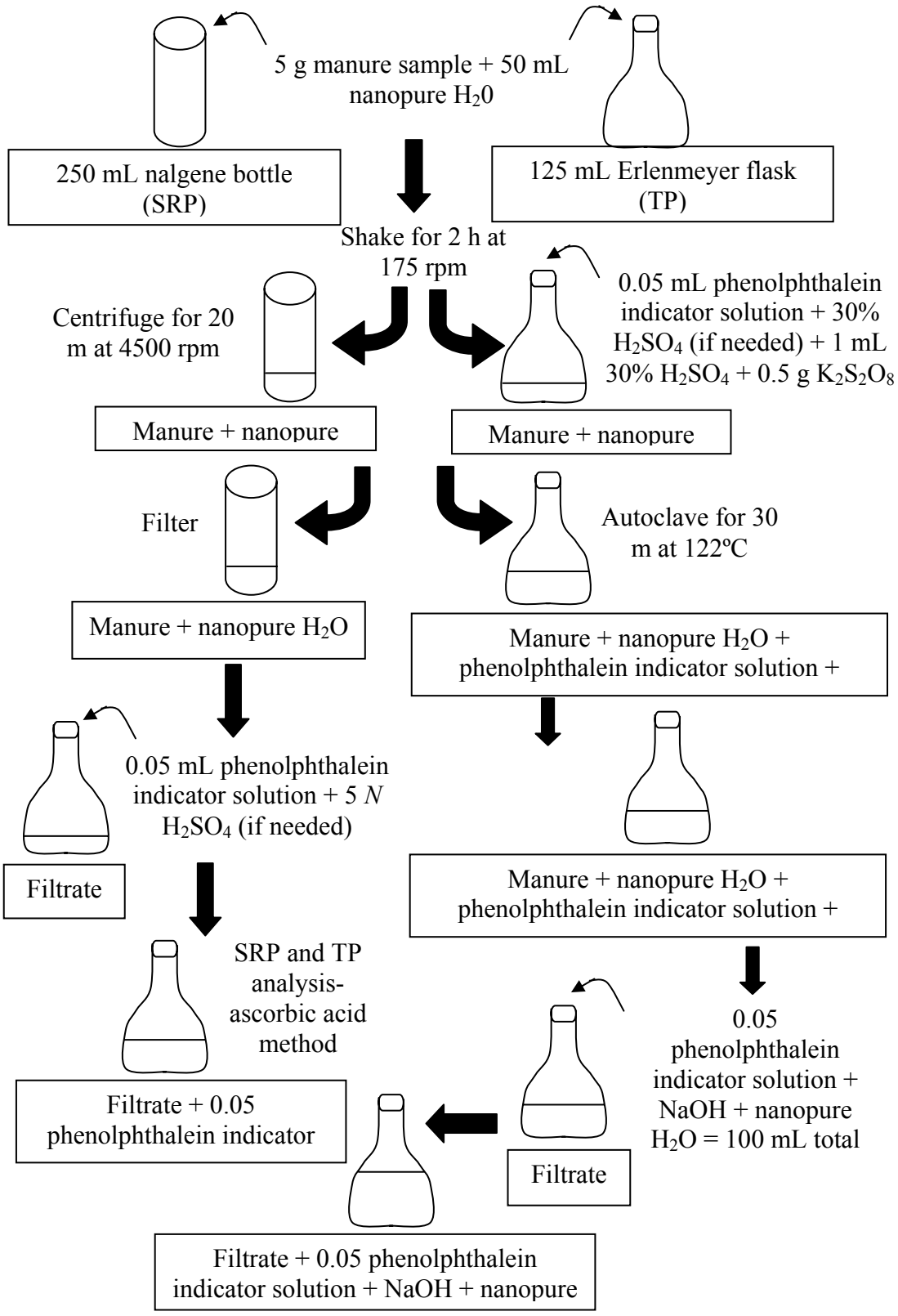


Figure 2.7. Flow diagram of the steps involved in TP and SRP sample preparation (Pierzynski 2000; American Public Health Association 1998).

The manure sample was first prepared by adding approximately 5 g of manure to 50 mL of nanopure water in a 250 mL nalgene bottle. The mixture was then shaken in a Max^{Q} 400 Shaker (Barnstead Lab Line, model SHKE4000-5) at 175 rpm for 2 hours followed by centrifuging (Thermo IEC. Marathon 21000R using a 4-place swinging bucket rotor (04-976-006) 4x250 mL) at 4,500 rpm or 3,760 relative centrifugal force (rcf) for 20 minutes. The supernatant was then vacuum filtered through a 0.45 µm membrane. The above procedures were obtained from Pierzynski (2000), but were then adapted to fit the needs of this project.

The filtrate was transferred to a 125 mL Erlenmeyer flask, and 0.05 mL of phenolphthalein indicator solution was added to determine if a red color developed. If a red color developed, a 5 *N* sulfuric acid (H_2SO_4) solution was added to lower the pH and discharge the color (American Public Health Association 1998, 4-147). The filtrate was then analyzed for SRP by the ascorbic acid method, as described under "SRP and TP analysis."

Standard/Solution/Reagent Preparation. Standards were first prepared in order to construct a standard curve. To do so, the stock solution was prepared by dissolving 0.4394 g of dry potassium phosphate (KH_2PO_4) in 100 mL of nanopure water (Doyle n.d.). Four different volumes; 20, 40, 60, and 80 µL of the stock solution were diluted to 100 mL of UHP in 100 mL volumetric glassware, resulting in standard solutions with concentrations of 200, 400, 600, 800 µL P/L, respectively. A 15 percent (w/w) H_2SO_4 solution was prepared by adding 77.7 g of 96.5 percent sulfuric acid to nanopure water to reach a total volume of 500 mL. A 5 *N* H_2SO_4 solution was prepared by adding 61.25 g of 96.5 percent H_2SO_4 to nanopure water to reach a total volume of 250 mL. A 30

percent (w/w) H_2SO_4 solution was prepared by dissolving 155.4 g of 96.5 percent H_2SO_4 to nanopure water to reach a total volume of 500 mL.

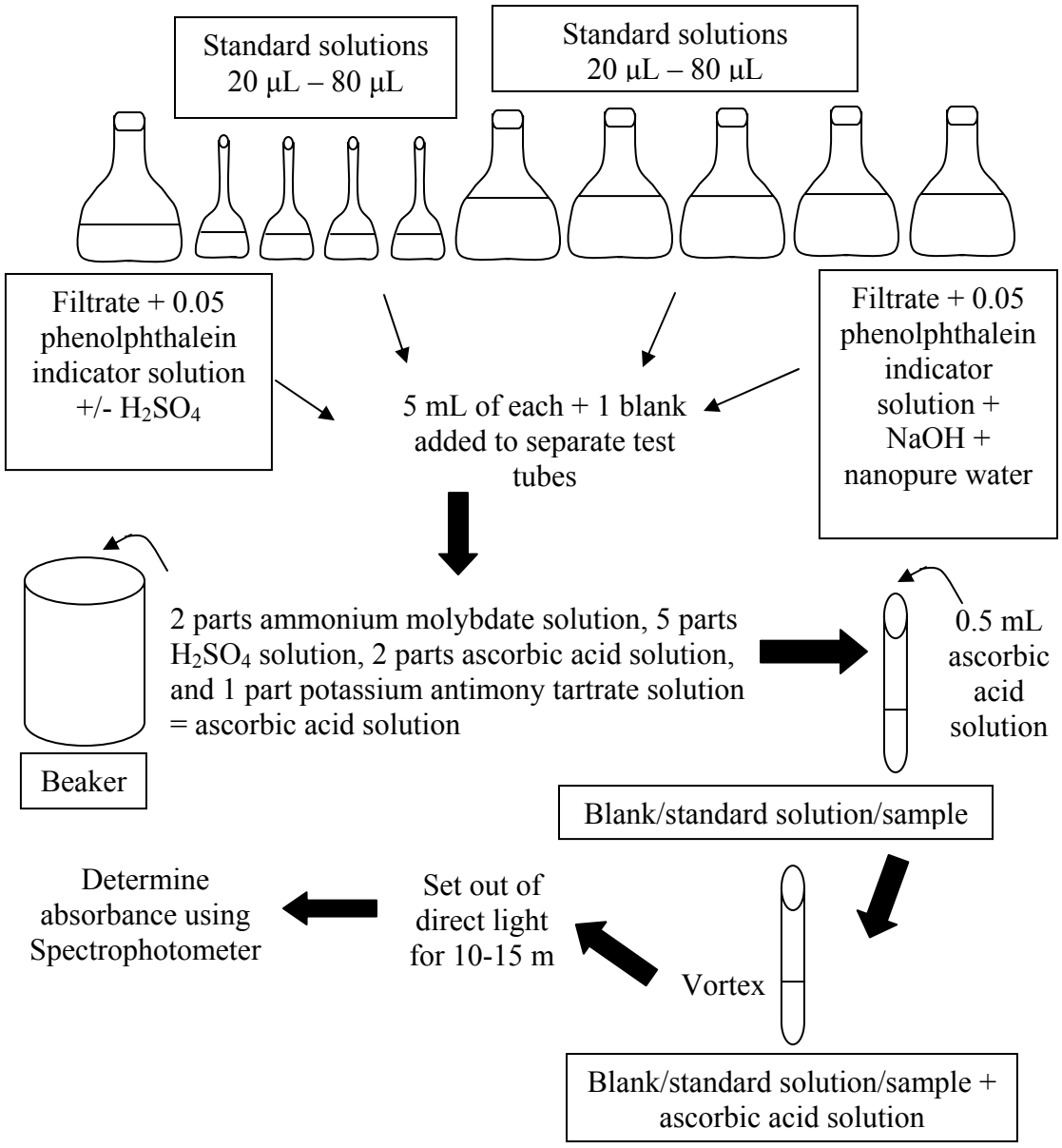


Figure 2.8. Flow diagram of the steps involved in TP and SRP analysis (Doyle n.d.; American Public Health Association 1998).

Next, the reagents were prepared for sample analysis. An ammonium molybdate solution was prepared by dissolving 6.0 g of ammonium molybdate ($(NH_4)_5Mo_7O_{24}\cdot 4H_2O$) in 200 mL of nanopure water. The ascorbic acid ($C_6H_8O_6$) solution was prepared by dissolving 2.7 g of ascorbic acid in 50 mL of nanopure water. The potassium antimony tartrate ($K_2[Sb_2(C_4H_4O_6)_2]\cdot 3H_2O$) solution was prepared by dissolving 0.272 g of potassium antimony tartrate in 200 mL of nanopure water (Doyle n.d.). The ascorbic acid solution must be prepared immediately before the mixed reagent is made, whereas the remaining three reagents may be prepared ahead of time.

The four reagents were combined to make the mixed reagent, which was made up of 2 parts of the ammonium molybdate solution, 5 parts of the sulfuric acid solution, 2 parts of the ascorbic acid solution, and 1 part of the potassium antimony tartrate solution (Doyle n.d.). The ammonium molybdate solution, ascorbic acid solution, and potassium antimonyl tartrate solution were stored in 250 mL nalgene bottles in the refrigerator. The sulfuric acid solution was stored in a sealed flask under the fume hood. All solutions could be stored indefinitely except for the ascorbic acid solution and potassium antimonyl tartrate solution, which expire after 1 week and 6 months, respectively (Doyle n.d.; American Public Health Association 1998, 4-147)

Persulfate Digestion Method

Sample Preparation. Approximately 5 g of the manure sample and 50 mL of nanopure water were placed in a 125 mL Erlenmeyer flask. For measuring TP in diluted and pretreated dairy cow manure 8 samples were used, whereas 12 samples were used, 2 subsamples of each fermentor bottle, for fermentation. The mixture was then shaken in a

Max^{Q} 400 Shaker (Barnstead Lab Line, model SHKE4000-5) at 175 rpm for 2 hours. The above procedures were adapted from Pierzynski (2000) in order to stay in tune with those procedures used to prepare manure for SRP analysis.

Following centrifuging, 0.05 mL phenolphthalein indicator solution, 1 mL of 30 percent (w/w) H_2SO_4, and 0.5 g potassium persulfate ($K_2S_2O_8$) were added (American Public Health Association 1998, 4-143). If a red color developed after the addition of the phenolphthalein indicator solution, 30 percent H_2SO_4 was added dropwise to each manure sample to discharge the color (American Public Health Association 1998). This solution was then cooked in an autoclave (Market Forge Sterilmatic, Block Scientific, Englewood, New Jersey) for 30 minutes at a temperature of approximately 122ºC. Once adequate time allowed for the solution to cool, it was filtered through a 0.45 µm membrane filter using a vacuum. Phenolphthalein indicator solution (0.05 mL) was added followed by a varying volume of 1 *N* sodium hydroxide (NaOH) needed to neutralize the solution to a faint pink color. A final volume of 100 mL was achieved with the addition of nanopure water (American Public Health Association 1999, 4-143, 4-144).

Total phosphorus was then determined by the ascorbic acid method, as described under "SRP and TP analysis." Fifty mL of nanopure water (the blank) and each phosphorus standard solution were also carried through the persulfate digestion procedure, and a separate standard curve from that of SRP was generated. The various H_2SO_4 solutions and NaOH were stored in volumetric flasks under the fume hood indefinitely.

SRP and TP Analysis

Five mL of each manure sample, standards, and a blank were transferred to test tubes. The blank was comprised of nanopure water that had gone through the persulfate digestion method as well. Then, 0.5 mL of the ascorbic acid solution was added to the blank, standard solutions, and samples (Doyle n.d.). Duplicates of the blank, standard solutions, and samples were made in order to account for the light tea color of the manure samples. Only 2 parts of the ammonium molybdate solution, 5 parts of the 15 percent H_2SO_4, and 3 parts nanopure water were mixed and added to the duplicates (American Public Health Assocation 1998, 4-147). The samples, standards, and blanks were then vortexed to thoroughly mix the contents and were set out of direct light for 10 to 15 minutes (Doyle n.d.; American Public Health Association 1998). Approximately 4 mL of the samples, standards, and blanks were poured into 1-centimeter (cm) glass cuvettes. Within at least 15 minutes, but no longer than 2 hours, of the ascorbic acid solution addition (Doyle n.d.), the absorbance of each standard and sample was determined by using a Spectrophotometer (Beckman Instruments, DU Series 500) at 880 nanometers (nm) (American Public Health Association 1998).

The analysis for determining SRP and TP in the dairy cow manure filtrate (batch 1) indicated that there was not a difference between those samples treated with 2 of the 4 mixed reagent solutions and those treated with all 4. Thus, the samples were treated with all 4 mixed reagent solutions for SRP and TP analysis in batches 1, 2, and 3.

Gas Analysis

The gas samples were analyzed with a GC using a packed column (Supelco-2390, J&W Scientific) and a thermal conductivity detector (TCD), and using helium as a

carrying gas. Once the GC was ready for use, a standard composed of 10.06 percent CH_4, 29.99 percent CO_2, and 49.94 percent N_2 (Airgas) was first injected into the GC gas port to calibrate the detector and establish residence times (Thanakoses, Mostafa, and Holtzapple 2003, 531). If subsequent samples presented shifted retention times, the analysis was repeated until the column stabilized.

Liquid Analysis

Upon the day of analysis, the liquid samples were thawed and centrifuged another time. Samples for GC (Agilent model 6890) analysis were prepared by mixing 1 mL of the centrifuged liquid sample with 1 mL of 1.162 g/L of 10-mM 4-methyl-n-valeric acid (an internal standard) and 1 mL of 3-M phosphoric acid (Thanakoses, Mostafa, and Holtzapple 2003, 531). The samples were acidified with phosphoric acid to protonate the acids and thus release them as volatiles in the GC injection port. The external standard consisted of a volatile acids standard mix solution (Matreya Inc. catalogue no. 1075). The GC used a capillary column (J&W Science model DB-FFAP) and FID detector, with helium as a carrying gas, and the results were integrated by GC chemstation V8.0 software. As a result, the concentrations of acetic, propionic, butyric, and heptanoic acids present as the manure fermented were determined.

Data Analysis

The results of batches 1, 2, and 3 were analyzed by both one and two-factor analysis of variance (ANOVA). The results of batch 1 required only the use of one-factor ANOVA because there was only one source of effect on the data (Turner and Thayer 2001, 35). However, the two-factor ANOVAs were needed to investigate the sources of

effect on the fermentation results of batch 2 and the pretreatment results of batch 3. Microsoft Excel was used to perform the one-factor ANOVA, while the two-factor ANOVAs were calculated by hand according to Turner and Thayer (2001, 53-70, 166-167) and Mickey, Dunn, and Clark (2004, 164). Refer to tables 2.3 to 2.5 for a list of the results that were analyzed and the appropriate ANOVA test.

Table 2.3. The result tested (DW=dry weight, AC=ash content, VS=volatile solid, TA=total acid, AA=acetic acid, L=limestone, B=bromoform, BP=before pretreatment, AP=after pretreatment, AF=after fermentation) and the appropriate ANOVA test for batch 1.

Result Tested	BP	AP	AF (60ºC)	AF (40ºC)	1 / 2-Way ANOVA
SRP, DW, AC, VS	X	X			1
	X		X		1
	X			X	1
		X	X		1
		X		X	1
SRP, DW, AC, VS, TA, AA, CO_2, pH, L, B			X	X	1

Table 2.4. The result tested (DW=dry weight, AC=ash content, VS=volatile solid, TA=total acid, AA=acetic acid, L=limestone, B=bromoform, P=pretreatment, BP=before pretreatment, AP=after pretreatment, AF=after fermentation) and the appropriate ANOVA test for batch 2.

Result Tested	BP	AP	AF (1-3)	AF (1B-3B)	AF (1P-3P)	AF (1PB-3PB)	1 / 2 - Way ANOVA
SRP, DW, AC, VS	X	X					1
	X		X				1
	X			X			1
		X			X		1
		X				X	1
SRP, DW, AC, VS, TA, AA, CO_2, CH_4, pH, L, B			X	X	X	X	2

Table 2.5. The result tested ((DW=dry weight, AC=ash content, VS=volatile solid, TA=total acid, AA=acetic acid, L=limestone, B=bromoform AP=after pretreatment, a=100ºC, 10% lime, + air, b=100ºC, 5% lime, +air, c= 100ºC, 10% lime, no air, d=100ºC, 5% lime, no air, e=40ºC, 10% lime, + air, f=40ºC, 5% lime, +air, g= 40ºC, 10% lime, no air, and h=40ºC, 5% lime, no air) and the appropriate ANOVA test for batch 3.

Result Tested	BP	AP a	AP b	AP c	AP d	AP e	AP f	AP g	AP h	1 / 2-Way ANOVA
SRP, DW, AC, VS	X	X								1
	X		X							1
	X			X						1
	X				X					1
	X					X				1
	X						X			1
	X							X		1
	X								X	1
		X	X	X	X					2
						X	X	X	X	2
		X		X		X		X		2
			X		X		X		X	2
		X	X			X	X			2
				X	X			X	X	2

CHAPTER THREE

Results

Effects of Lime Pretreatment and Fermentation on SRP

The pretreatment and fermentation steps of the MixAlco process successfully reduced the amount of orthophosphate in the reacting fluids of dairy cow manure, which for purposes of simplicity is referred to as SRP in dairy cow manure. Figure 3.1 presents results from fermentation experiments comparing SRP levels before and after pretreatment and after fermentation at 60ºC and 40ºC. It can be seen that lime pretreatment resulted in a dramatic reduction in SRP and that fermentation, at both temperatures, appeared to reduce the SRP levels even further.

Production of organic acids in a digester requires suppression of CH_4 production. An experiment was carried out to compare the SRP reduction achieved in fermentations either producing CH_4 (methanogenic fermentation) or suppressing CH_4 production (acidogenic digestion). This experiment also tested the reduction of SRP resulting from the fermentation carried out without lime pretreatment. Figure 3.2 presents the results from this experiment. As was found in batch 1, lime pretreatment significantly reduced SRP levels, and fermentation of pretreated manure also further reduced SRP levels. Though the high scatter in the pretreated samples reduces the certainty of these results. Yet, fermentation without pretreatment did reduce SRP levels found in the filtrate from nonpretreated cow manure. Pretreatment and fermentation, as opposed to fermentation alone, appears to have made a difference in SRP levels. In both fermented manure and

pretreated and fermented manure, the methanogenic bottles contained lower levels of SRP than the acidogenic bottles.

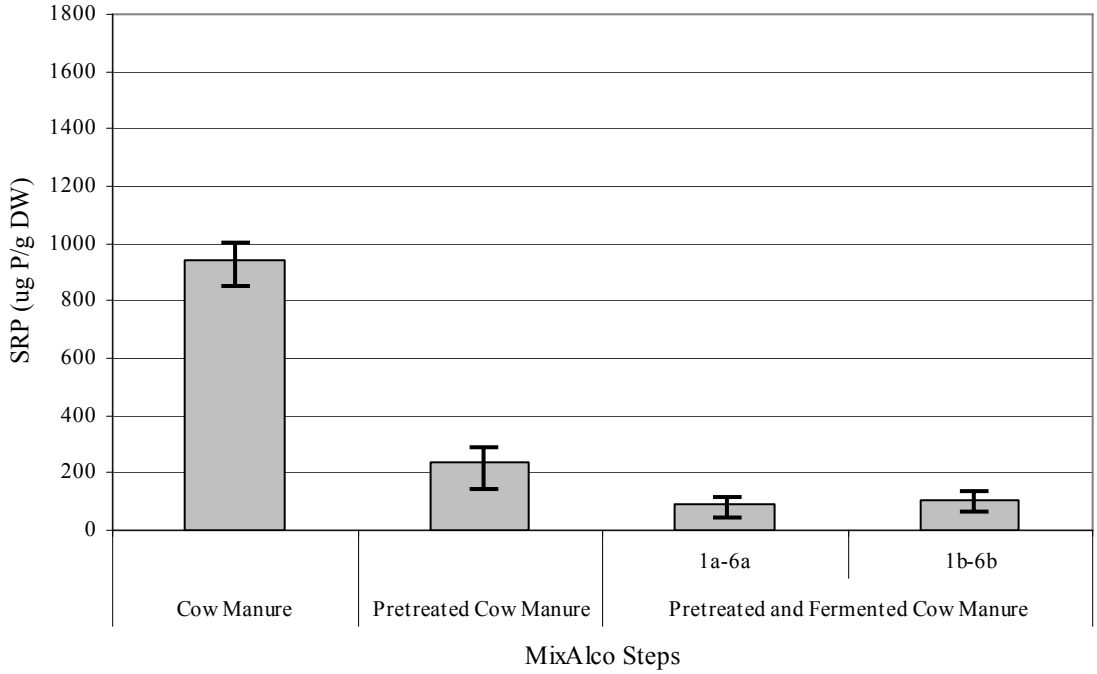


Figure 3.1. SRP concentrations (μg/g DW) found in the dairy cow manure filtrate (batch 1) before and after lime pretreatment (0.1g $Ca(OH)_2$/1.0g DW) and fermentation (CH_4 inhibition, 60ºC (bottles 1a-6a) and 40ºC (bottles 1b-6b)). Error bars represent + and – 1 standard deviation.

Effect of Lime Pretreatment Conditions on SRP

It has been proposed that lime pretreatment can be carried out at temperatures ranging from ambient to 150°C, and with or without aeration of the system (Kim and Holtzapple 2006, 584; Chang et al. 2001b, 1, 3). An experiment was conducted to determine the relative effectiveness of SRP reduction under different pretreatment conditions. The conditions tested included pretreatment at 40ºC and 100ºC, with either 5 or 10 percent lime addition, and with or without aeration. The error bar on the analysis of the initial sample is very large, which makes it difficult to verify the effectiveness of each

applied treatment. Significant differences can be seen however, between the different treatments.

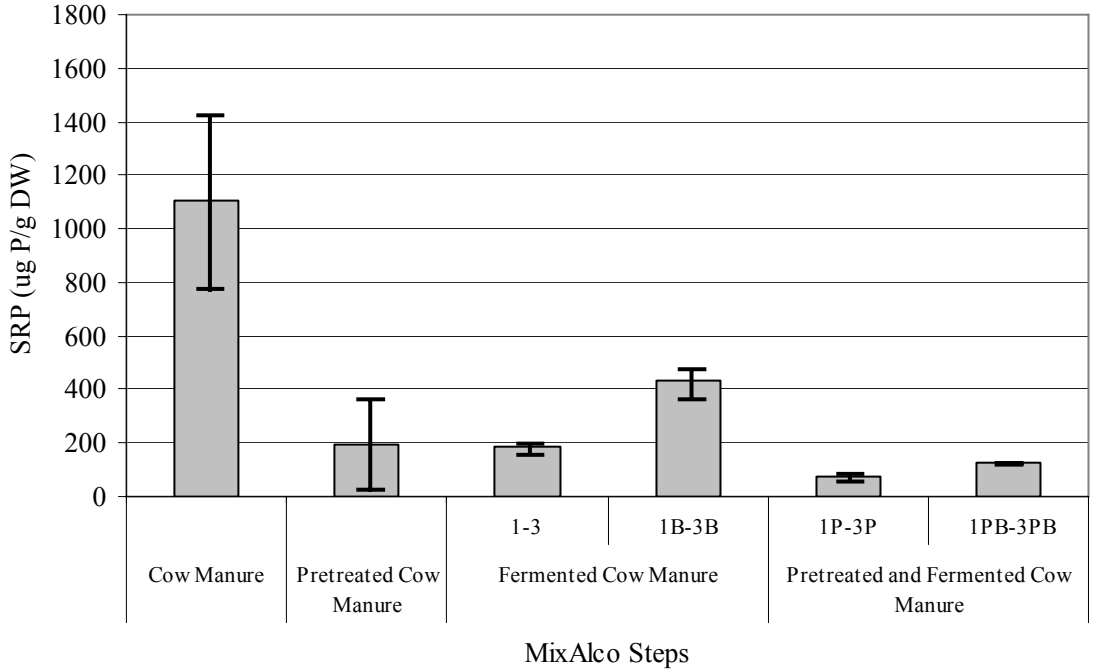


Figure 3.2. SRP concentrations (μg/g DW) found in the dairy cow manure filtrate (batch 2) before and after lime pretreatment (0.1g $Ca(OH)_2$/1.0g DW) and/or fermentation (no pretreatment and no CH_4 inhibition (bottles 1-3), no pretreatment and CH_4 inhibition (bottles 1B-3B), pretreatment and no CH_4 inhibition (bottles 1P-3P), and pretreatment and CH_4 inhibition (bottles 1PB-3PB), all operating at 40ºC). Error bars represent + and – 1 standard deviation.

Effect of Temperature on Acid Production

Batch 1 compared the acid production of fermentations carried out at 60ºC and 40ºC. It was observed that the total acid production was similar at both temperatures. Figure 3.4 illustrates the trend of acid concentration over time for a 30-day experiment. The acid concentrations increased in the beginning, appeared to level off and drop and then rise again by the end of fermentation. There was a decrease in acid concentration at day 12 and 18 for bottle 6a and at day 18 for bottle 1b. Figure 3.5 presents final acid

concentration results for each of the fermentation bottles in batch 1. Temperature appeared to affect the relative concentration of acetic acid (C2) versus longer chain acids. By day 30, most of the total acid concentration produced in the bottles fermenting at 60ºC (series "a") was acetic acid. The C2 acid concentrations were lower for the 40ºC fermentation (series "b") and represented a smaller percentage of the total acids than those produced at 60ºC. Fermentation at 60ºC appeared to yield more consistent results for final acid concentration than did the fermentations at 40ºC.

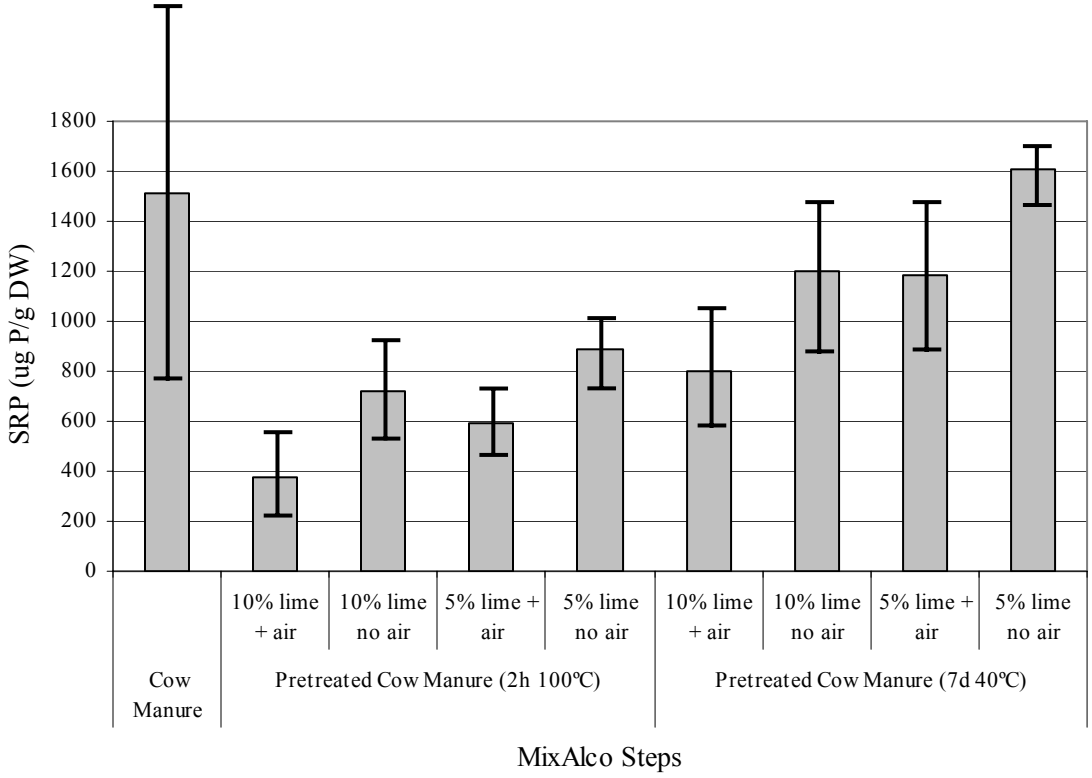


Figure 3.3. SRP concentrations (µg/g DW) found in the dairy cow manure filtrate (batch 3) before and after lime pretreatment (0.05 g $Ca(OH)_2$/1.0 g DW or 0.10 g $Ca(OH)_2$/1.0 g DW, with and without the circulation of air). Error bars represent + and – 1 standard deviation.

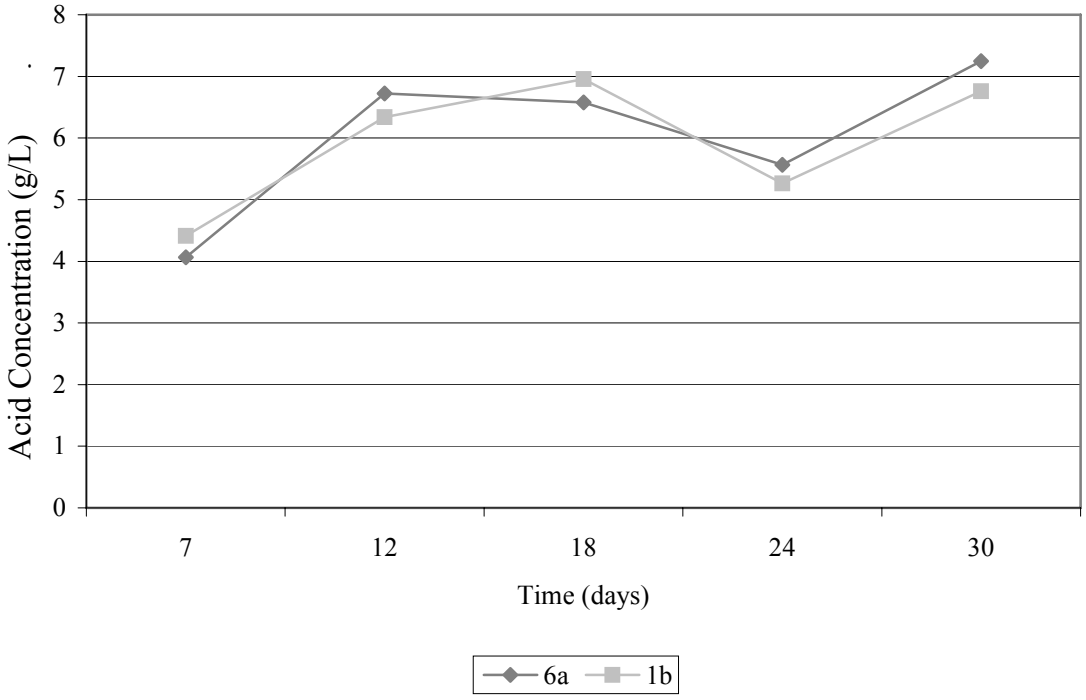


Figure 3.4. Total acid concentrations (g/L) over time for bottles 6a (60ºC) and 1b (40ºC) (batch 1).

Effect of Temperature on Gas Production

Figure 3.6 is the result of daily accumulation of CO_2 in the series "a" bottles with CH_4 inhibition, while the series "b" bottles, also with CH_4 inhibition, are shown in figure 3.7. The production of CO_2 in series "a" and "b" bottles appear to vary as a result of the temperature difference. The cumulative CO_2 curves, as seen in figures 3.6 and 3.7, are different in that the series "a" bottles produced more CO_2 at the beginning of the experiment than the series "b" bottles. In Figure 3.6, the most CO_2 production occurred at days 2 and 3, while the most CO_2 production did not occur until about day 6 in figure 3.7. The gaps in the data represent either a poor sample injection to the GC or that the chromatograms appeared unrealistic. In general, by day 30, the series "a" bottles appear to have produced more CO_2 than those in series "b." In addition, CO_2 production in the

series “a” bottles appear to have stabilized by day 14 or 15, whereas the series “b” bottles did not appear to stabilize until day 21.

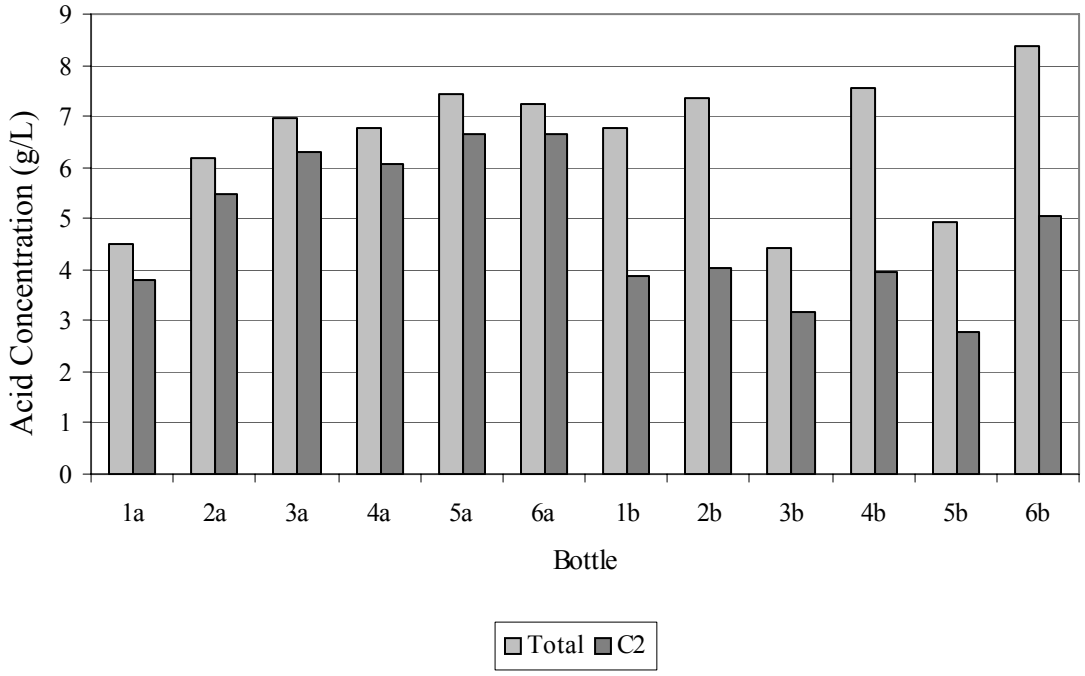


Figure 3.5. Total acid concentrations (g/L) at day 30 per bottle (batch 1).

Figures 3.8 and 3.9 show the associated daily CH_4 accumulation for series” a” and “b.” As expected, batch 1, both series “a” and “b” bottles, produced very little CH_4, if any. Despite CH_4 inhibition, bottles 1a and 6b produced significantly greater amounts of CH_4 than the remaining bottles in both series. However, gas production was high for only 1 day, day 3 for bottle 1a and day 17 for bottle 6b. Methane concentrations in these particular bottles remained reasonably stable for the remainder of the experiment. The addition of bromoform, for the sole purpose of CH_4 inhibition, appeared to keep CH_4 volumes at or near 0 with only 1 bottle in each series reaching approximately 18000 and 6000 mL, respectively.

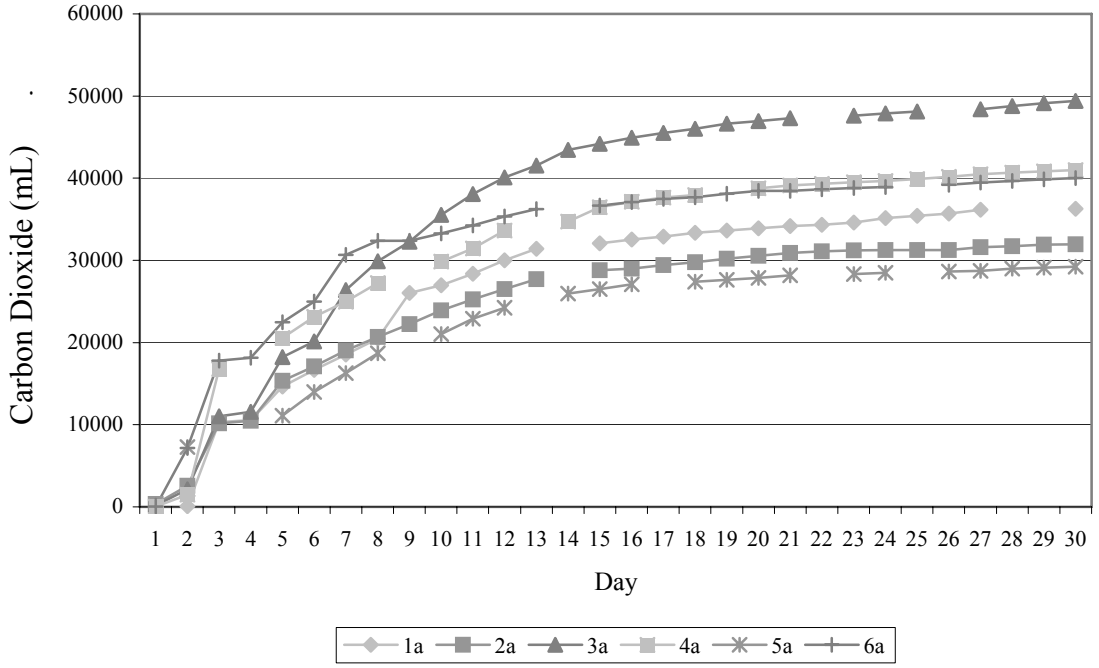


Figure 3.6. Cumulative CO_2 volume (mL) per day for bottles fermenting at 60°C (batch 1).

Effects of Pretreatment and Methane Inhibition on Acid Production

Batch 2 compared the effects of pretreatment and bromoform addition on the accumulation of organic acids. Bromoform inhibits the production of CH_4. Both variables resulted in changes in acid production. Figure 3.10 presents results from this experiment. Those bottles inhibited with bromoform (3B, 2PB) accumulated acids steadily over the 30-day period. The pretreated fermentation sample (2PB) benefited from a faster initial accumulation rate than the nonpretreated sample (3B). The bottles that were not inhibited with bromoform (1, 2P) both started growth by accumulating acids. After the acid concentrations had accumulated for a few days they then declined, as would be expected for a methanogenic culture converting organic acids to CH_4. As was observed in the acidogenic cultures, acid accumulation was more rapid for the

pretreated sample than the fermentation initiated without pretreatment. Figure 3.11 presents the final acid concentrations measured for each bottle in batch 2, where total and C2 acid concentrations are shown. It can be seen that the bottles receiving no pretreatment or bromoform accumulated little acid with no C2 acids at all. Bottles treated with bromoform did accumulate acids, about half of which were C2. Two of the uninhibited, but pretreated samples (1P and 3P) did appear to accumulate acids despite the lack of bromoform.

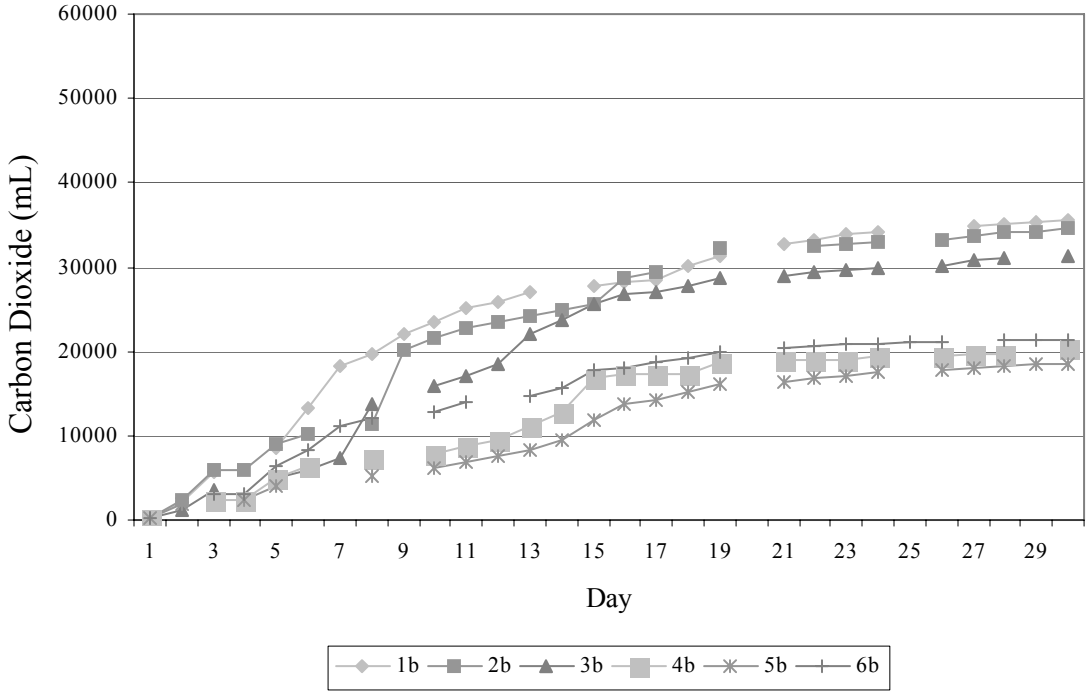


Figure 3.7. Cumulative CO_2 volume (mL) per day for bottles fermenting at 40°C (batch 1).

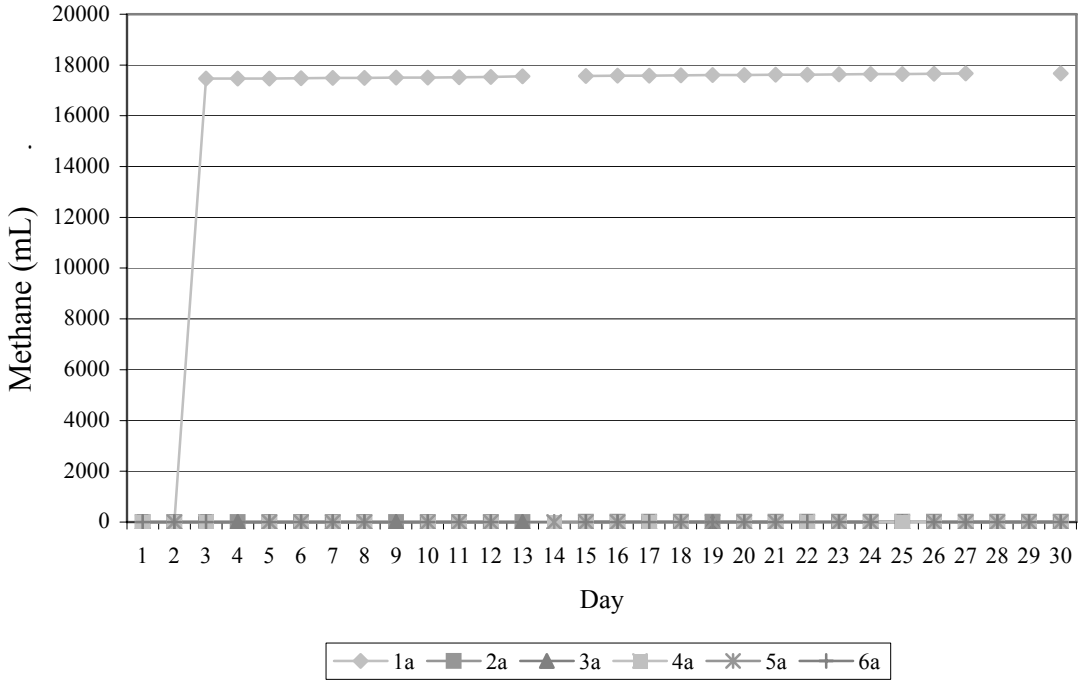


Figure 3.8. Cumulative CH_4 volume (mL) per day for bottles fermenting at 60°C (batch 1).

Effects of Pretreatment and Methane Inhibition on Gas Production

The production of CO_2 in batch 2 appeared to vary based upon whether or not bromoform was added to the samples. There does not appear to be a significant or consistent difference among those bottles that were and were not pretreated with lime. However, in figure 3.12, the bottles with the most CO_2 production were 2 nonpretreated bottles, and the bottles with the lowest CO_2 production were 2 pretreated bottles. In figure 3.13, the top 3 CO_2 producing bottles were those that were pretreated. Among the methanogenic bottles, the cumulative volume of CO_2 production by day 30 was higher in every bottle than in the acidogenic bottles, whereas the methanogenic bottles produced more CO_2 in the early stages of the experiment. Days 10 and 7 signify the days at which CO_2 production began to increase at a higher rate in figures 3.12 and 3.13, respectively.

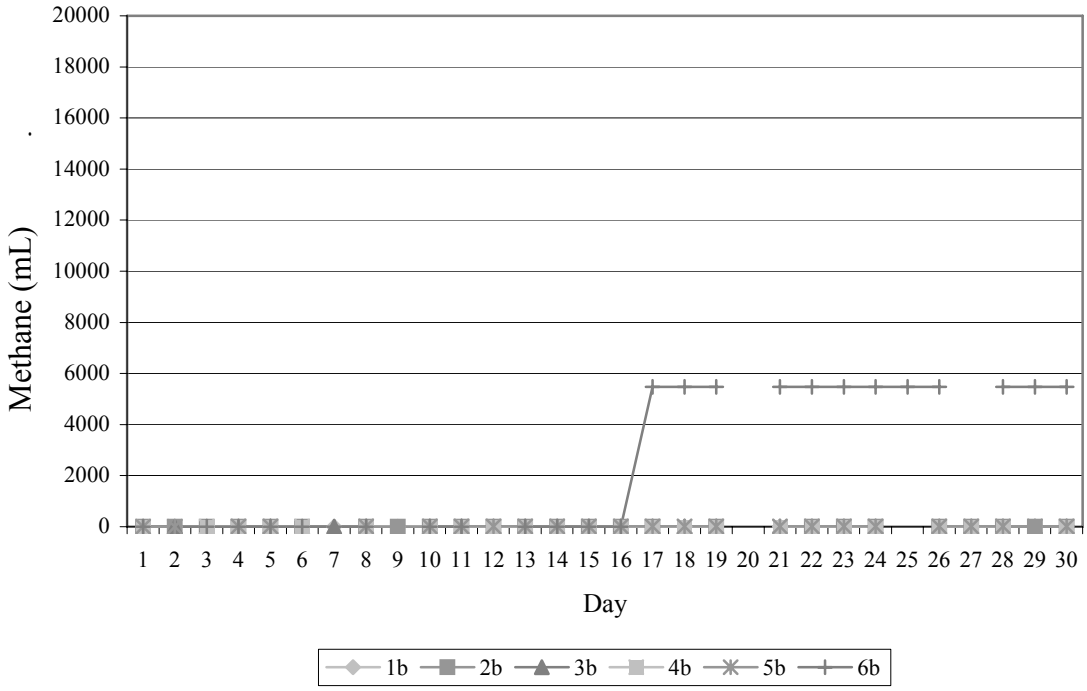


Figure 3.9. Cumulative CH_4 volume (mL) per day for bottles fermenting at 40°C (batch 1).

Methane production appears to begin to dramatically increase at day 10 in figure 3.14. Methane in figure 3.15 was inhibited, thus there is generally very little CH_4 production. Bottle 2PB was erratic in that it began to produce relatively significant amounts of CH_4 at day 13, followed by a lowered increase in production. With the exception of bottle 2B, by day 30 those pretreated methanogenic bottles had a lower CH_4 volume than those nonpretreated methanogenic bottles.

The pretreated acidogenic bottles in batch 2 (1PB-3PB) produced a significantly lower volume of CO_2 than those in batch 1 (1a-6a). In addition, CO_2 production dramatically increased at day 3 in batch 1 as compared to day 7 or 8 in batch 2.

pH Trends in Fermentation

For batches 1 and 2, the pH immediately dropped from an initial pH of around 7, as can be seen in figures 3.16 and 3.17. The pH began to slowly increase at day 3 until day 30. Each day, series "a" bottles operated at a lower pH than series "b" bottles. This may be indicative of the higher proportion of acetic acid in these fermentations, which has a lower pKa than the longer chain acids (Weast and Astle 1978, D202-D203). In batch 2, the pH curves of those nonpretreated bottles were different whereas the pH of those pretreated bottles responded more or less the same as in batch 1. By the end of the experiment, the bottles inhibited with bromoform registered lower pH levels than those that were uninhibited. This corresponds to the higher acid concentrations measured in these samples.

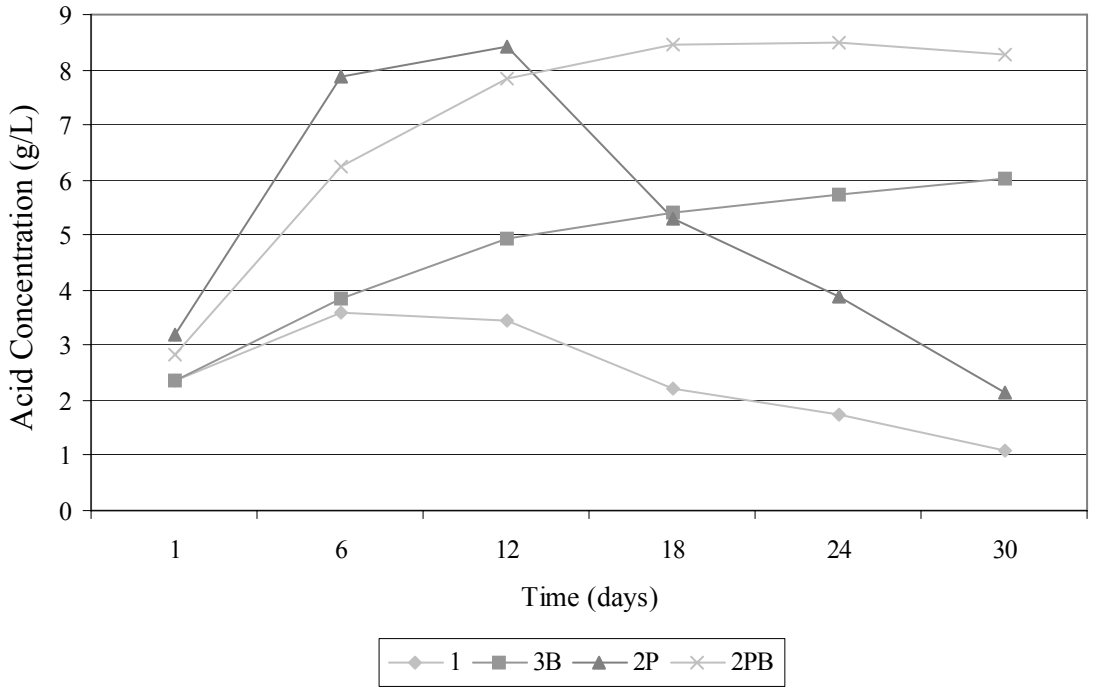


Figure 3.10. Total acid concentrations (g/L) over time for bottles 1 (no pretreatment, no bromoform), 3B (no pretreatment, with bromoform), 2P (with pretreatment, no bromoform), and 2PB (pretreatment and bromoform).

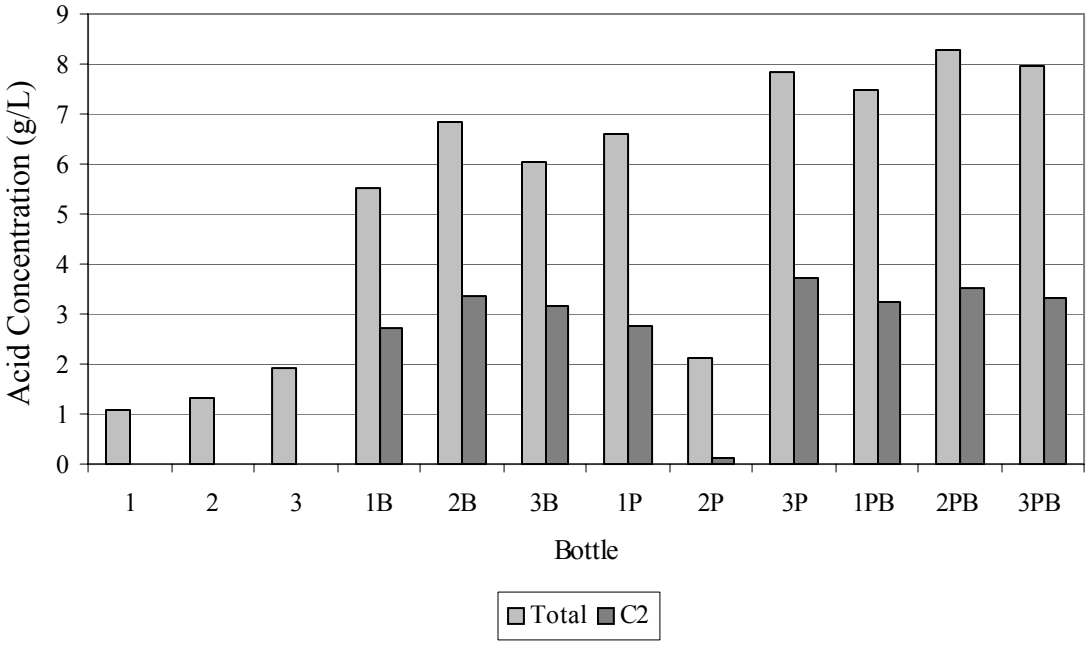


Figure 3.11. Total acid concentrations (g/L) at day 30 per bottle (batch 2).

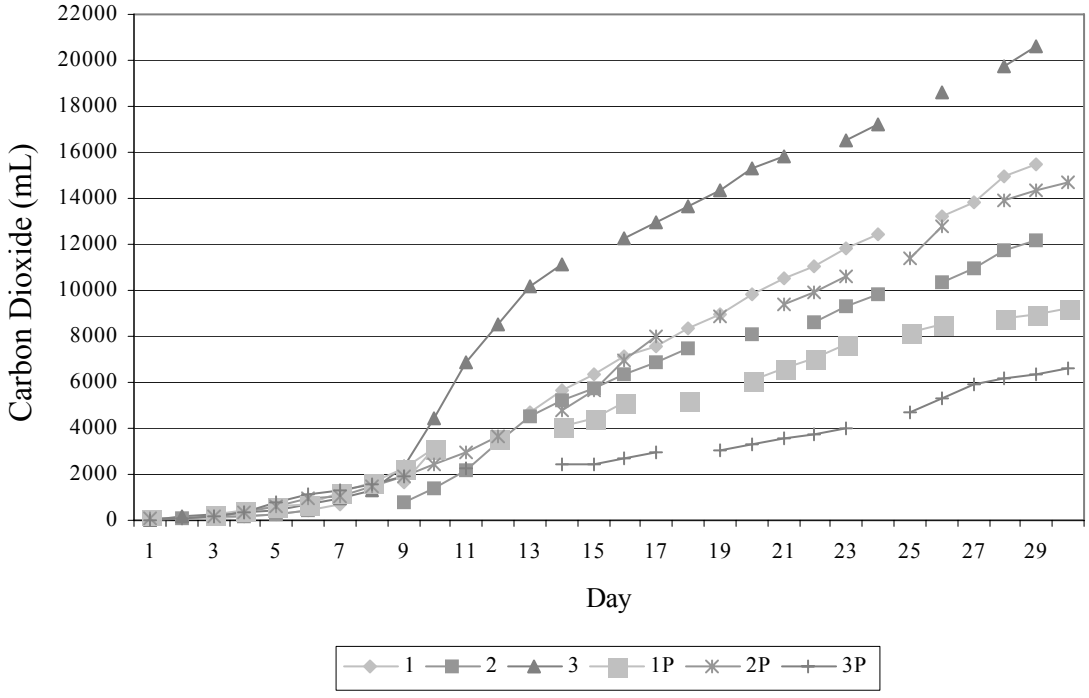


Figure 3.12. Cumulative CO_2 volume (mL) per day for bottles not inhibited with bromoform (batch 2).

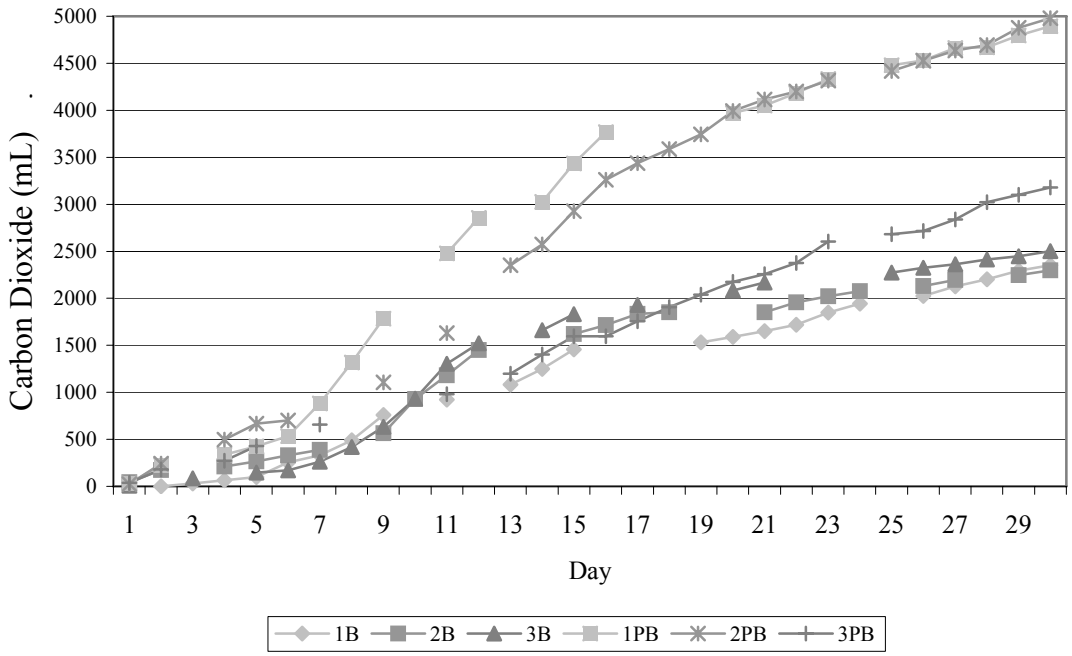


Figure 3.13. Cumulative CO_2 volume (mL) per day for bottles inhibited with bromoform (batch 2).

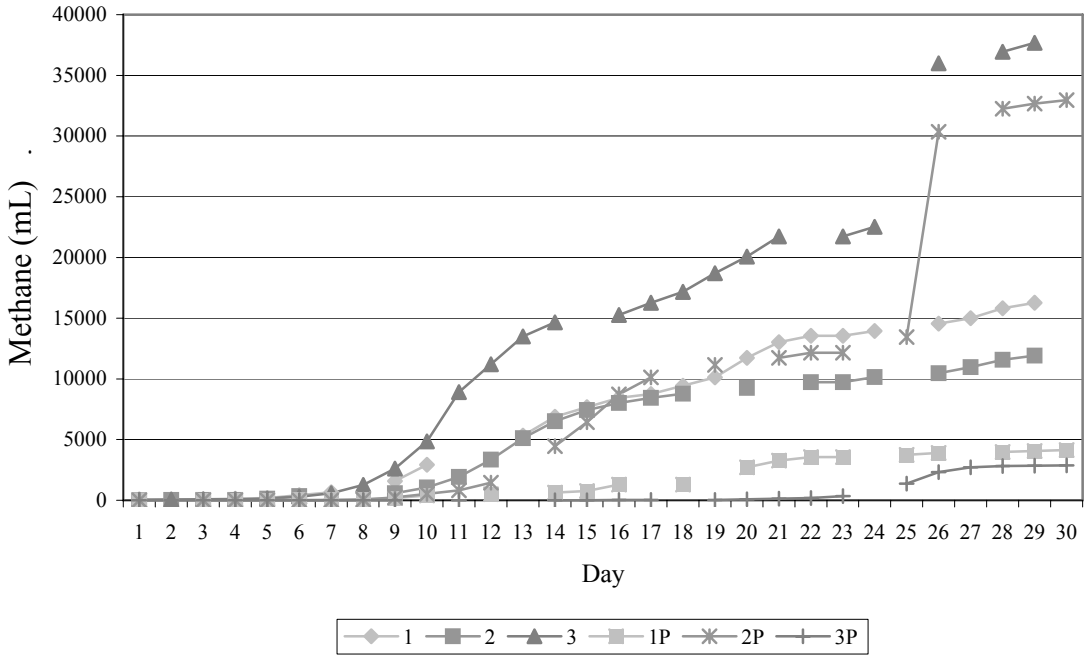


Figure 3.14. Cumulative CH_4 volume (mL) per day for bottles not inhibited with bromoform (batch 2).

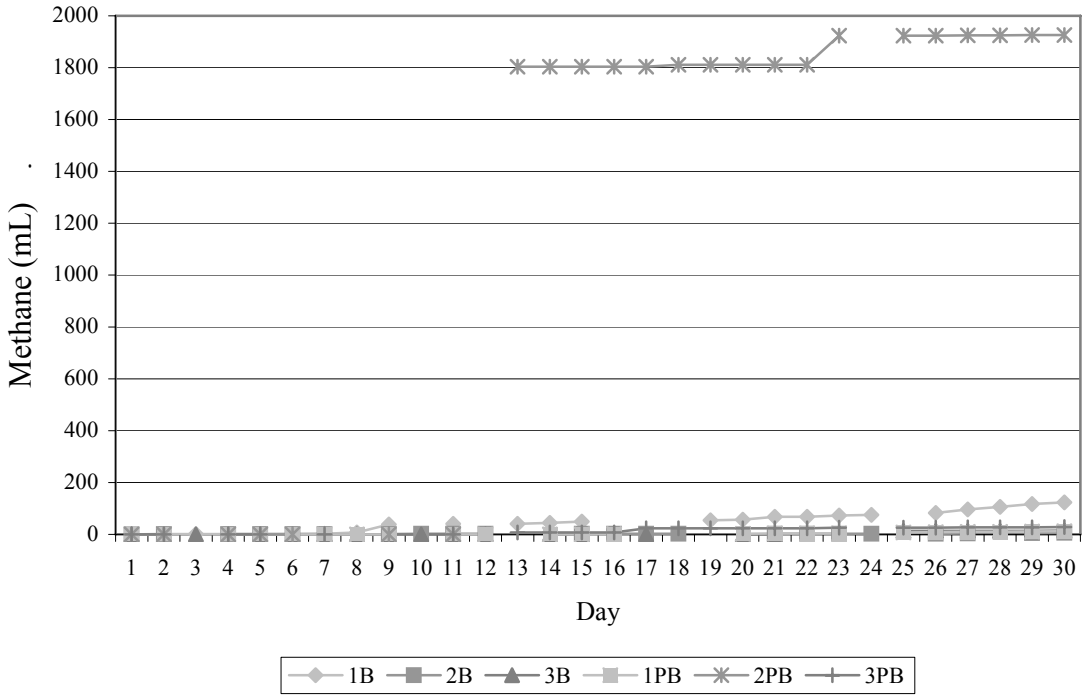


Figure 3.15. Cumulative CH_4 volume (mL) per day for bottles inhibited with bromoform (batch 2).

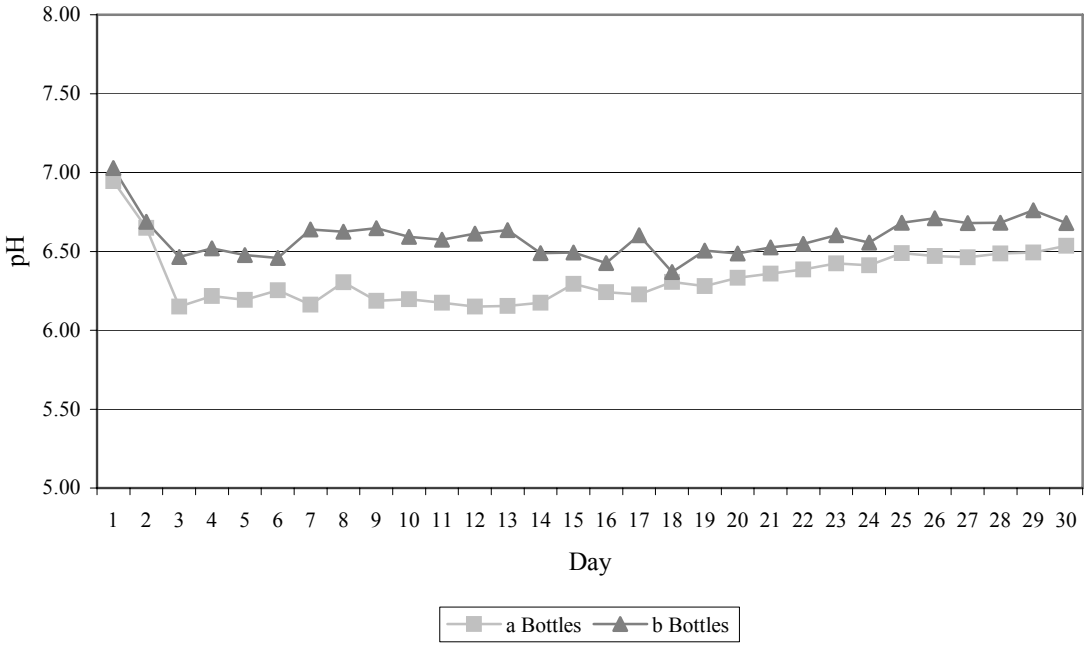


Figure 3.16. Average pH for 12 bottles, 6 fermenting at 60ºC (series "a") and 6 fermenting at 40ºC (series "b") (batch 1).

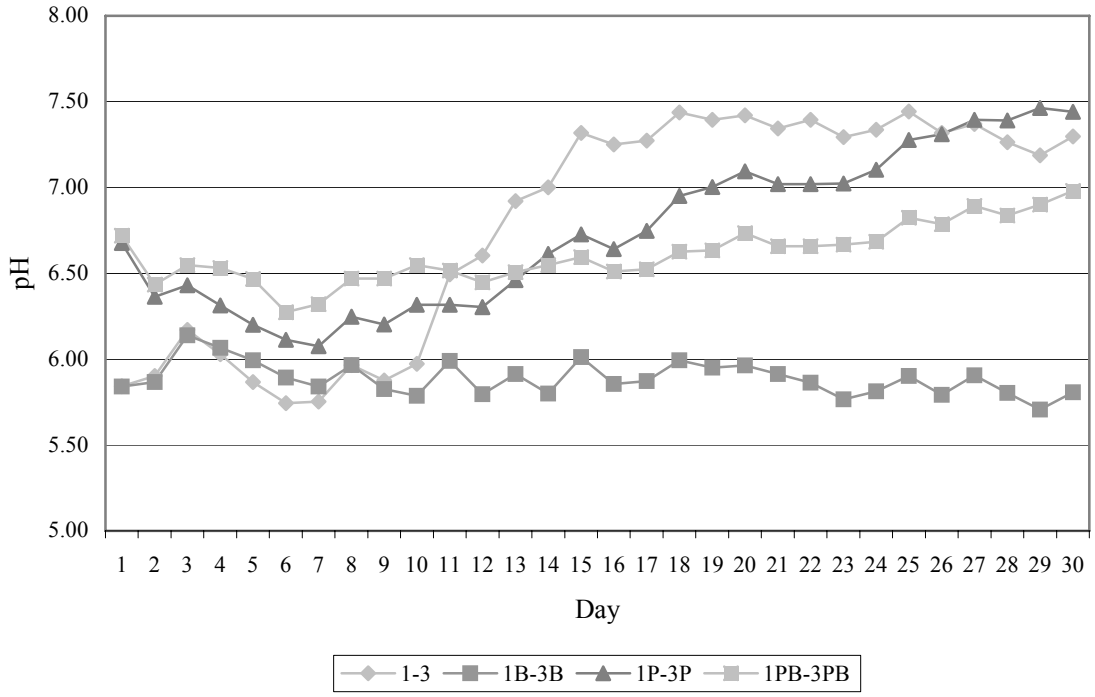


Figure 3.17. Average pH for 12 bottles, all fermenting at 60ºC, 6 of which were pretreated (P) and 6 of which were inhibited with bromoform (B) (batch 2).

Dry Weight, Ash Content, and Volatile Solids

The dry weight, ash content, and volatile solids were determined for dairy cow manure before and after pretreatment (figures 3.18, 3.19, and 3.20), and after fermentation (figures 3.18 and 3.19). The fermented cow manure in batch 1 had a higher dry weight than that found in cow manure before and after pretreatment. There appears to be no difference between the dry weights of the fermented manure, no matter the condition. In addition, the dry weight of cow manure and pretreated cow manure appear to be similar. In batch 3, the dry weight appears to have increased with 5% lime, and with and without air circulation at a pretreatment of 100ºC for 2 h, and decreased at a pretreatment of 40ºC for 7 d, with 5% lime, and with air circulation. The dry weight in

batch 2 increased after pretreatment and fermentation; however, due to the overlapping error bars, the differing fermentation conditions appear to have an inconclusive effect.

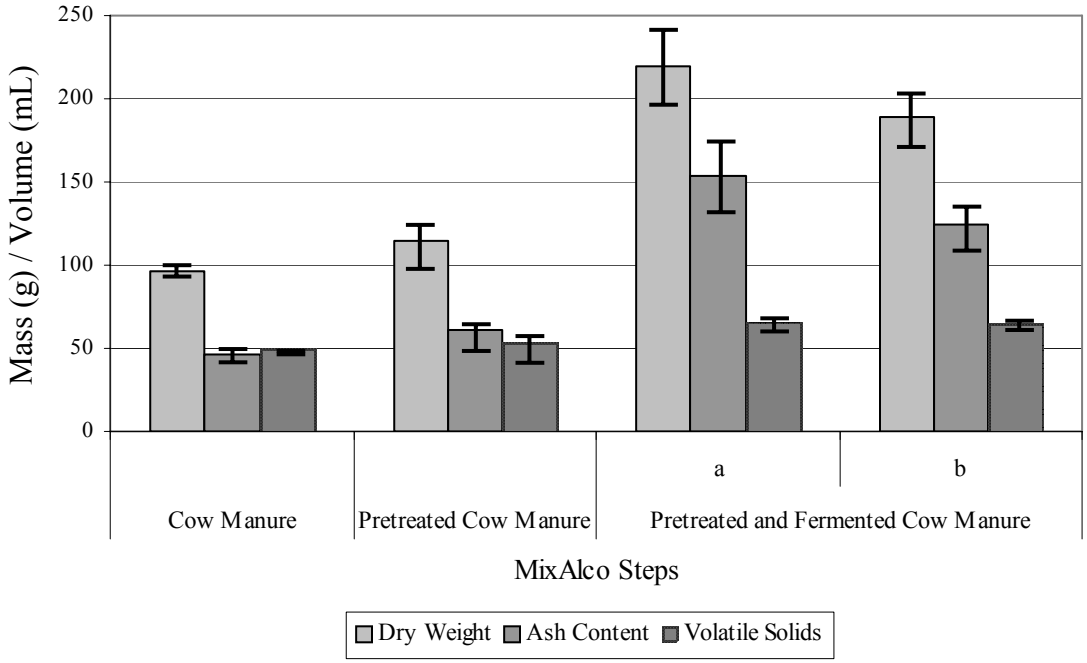


Figure 3.18. Dry weight, ash content, and volatile solids (g/mL) of dairy cow manure before and after pretreatment and fermentation (batch 1). Error bars represent + and –1 standard deviation.

The ash content in batch 1 increased from cow manure and pretreated cow manure to the fermented cow manure. There does not appear to be a difference in ash content before and after pretreatment, and among the series "a" and "b" bottles. Also, in batch 2 the ash content is similar before and after pretreatment and among the 4 varying fermenting conditions. In addition, the ash content increased in the fermented manure. The ash content of cow manure before pretreatment, as seen in Figure 3.20, appears to only be different from the ash content of cow manure pretreated at 100ºC for 2 h with both 10 and 5 % lime and air circulation.

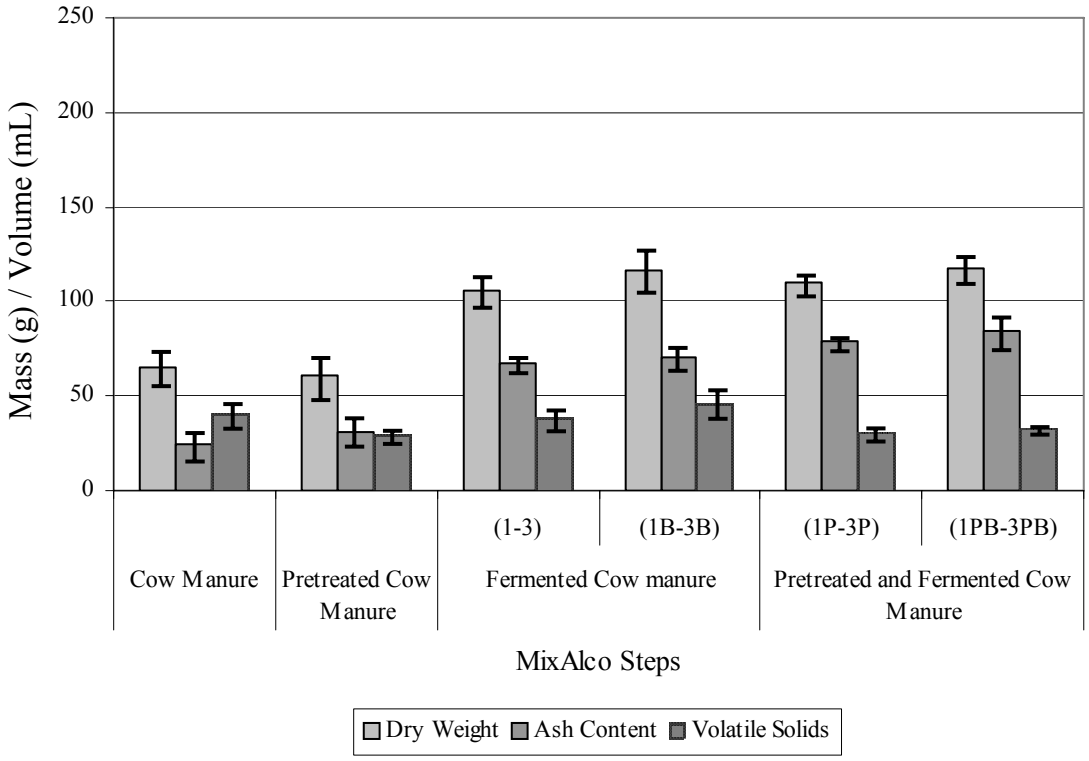


Figure 3.19. Dry weight, ash content, and volatile solids (g/mL) of dairy cow manure before and after pretreatment and after fermentation (batch 2). Error bars represent + and –1 standard deviation.

A volatile solid is the difference between the dry weight and the ash content. In almost every step, the volatile solids are the smallest portion of the dry weight. This is true for batch 3, and the majority of batches 1 and 2. In the first step of batches 1 and 2, the volatile solids fraction is larger than the ash content fraction. But, they are almost equal in the first step of batch 3.

There appears to be no difference among the volatile solids in batch 3. However, there may be a small increase from the volatile solids present in cow manure and pretreated cow manure (100°C for 2 h) with 5% lime and air circulation. As with the ash content in batch 1, there may only be a small increase in volatile solids between cow manure and fermented manure. There is not a difference between pretreated manure and

fermented manure, or among the fermented manure. In batch 2, there appears to be a slight decrease in volatile solids from cow manure to pretreated manure. In addition, there may be a slight difference between the bottles pretreated and fermented and the fermented bottles. Thus, pretreatment may have lowered the volatile solids content.

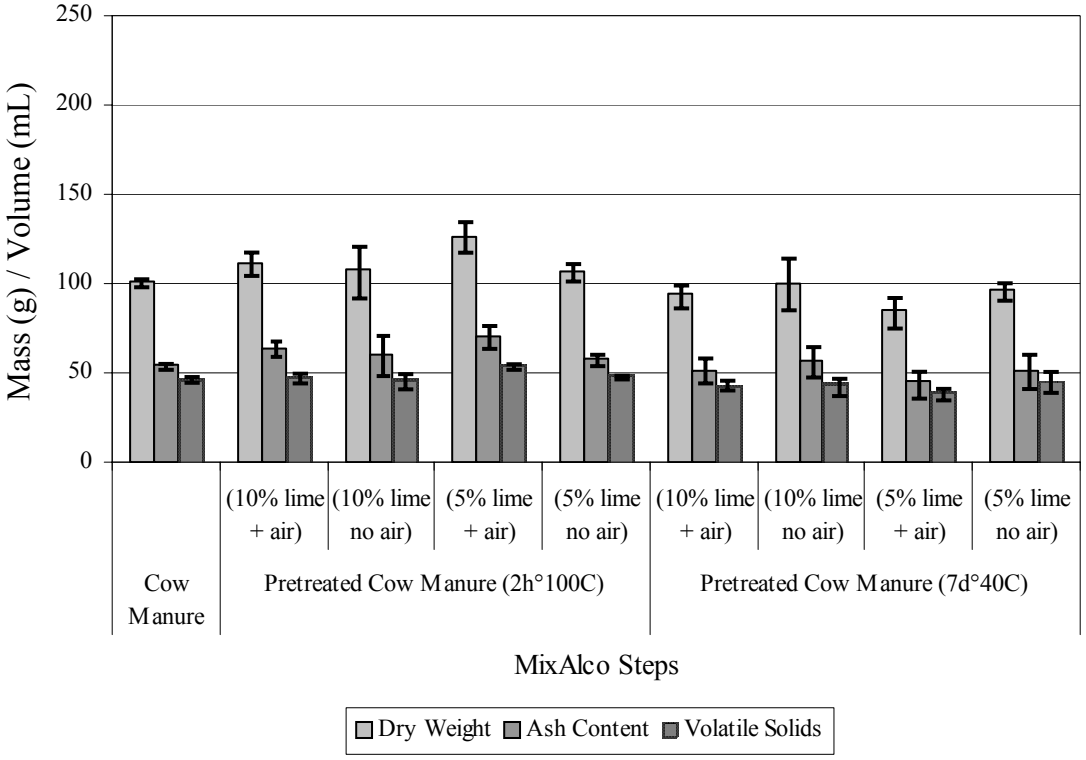


Figure 3.20. Dry weight, ash content, and volatile solids (g/mL) of dairy cow manure before and after pretreatment (batch 3). Error bars represent + and – 1 standard deviation.

CHAPTER FOUR

Discussion

Effect of Pretreatment Conditions on SRP

There is much evidence that lime will precipitate soluble forms of phosphorus into insoluble forms (Koschel 1997; van Loon and Duffy 2000, 359-360; Koutsoukos 2004; Lind 2002). This experiment provides more support for this statement. In batches 1, 2, and 3, after lime pretreatment there was a reduction in the amount of orthophosphate in the reacting fluids of dairy cow manure, which for purposes of simplicity is referred to as SRP in dairy cow manure.

Batch 1

Lime pretreatment of cow manure with a lime loading of 0.10 g/g DW (2 h 100ºC) resulted in an average reduction in SRP of 74 % (938.74 to 239.59 μg P/g DW).

Batch 2

Lime pretreatment of cow manure with a lime loading of 0.10 g/g DW (2 h 100ºC) resulted in an average reduction in SRP of 83 % (1104.22 to 191.62 μg P/g DW).

Batch 3

Lime pretreatment of aerated cow manure with a lime loading of 0.05 g and 0.10 g/g DW (2 h 100ºC) resulted in an average reduction in SRP of 61 and 75 % (1513.07 to 594.27 and 373.43 μg P/g DW), respectively. According to figure 3.3, there appears to

be a difference in SRP concentrations in the pretreated manure filtrate based upon aeration, lime loading, and temperature. In this experiment there was a large degree of scatter in the initial, nonpretreated SRP measurements. Because of the large standard deviation, most analyses that follow will be between the pretreated samples.

Aeration. There was evidence of a significant ($p < 0.01$) difference in SRP concentrations among the aerated pretreated bottles and nonaerated pretreated bottles. Lower concentrations in the aerated bottles may be the result of its ability of increased mixing associated with the bubbling of air through the sample, which would more effectively precipitate soluble forms of phosphorus.

Lime Loading. There were significant ($p < 0.05, 0.01$) differences among the bottles pretreated with varying amounts of lime. An increase in lime, from 0.05 to 0.10 g/g DW of manure, generally resulted in a reduction in SRP concentrations due to lime's precipitating ability.

Temperature. An increase in temperature, from 40ºC to 100ºC, also generally resulted in a significant ($p < 0.01$) reduction in SRP concentrations. The higher temperature enhanced manure digestibility, which likely enhanced lime's precipitating ability.

Batches 1, 2, and 3

Pretreatment for 2 h at 100ºC, at a lime loading of 0.10 g/g DW of manure, with aeration, had the greatest effect in terms of SRP reduction in batch 3. The only

pretreatment condition (2 h 100ºC) was successful at reducing SRP concentrations in both batches 1 and 2. However, SRP reduction in batch 2 was more successful than batch 1.

The SRP concentrations in batches 1 and 2 before pretreatment were more similar than that of batch 3. According to figure 2.3, batches 1 and 2 were collected near the entrance of the lagoon whereas batch 3 was collected close to the runoff ramp. As manure accumulates in the parlor, it is hosed into the holding lagoon. The floor of the lagoon is on an incline, thus the "fresh" manure is located closer to the lagoon entrance. As a result of this incline and of the constant hosing off of manure from the parlor to the lagoon, perhaps the more soluble forms of phosphorus were able to easily runoff into the containment section close to the runoff ramp and near the collection site of batch 3. The sampling location of batch 3 may explain the presence of a higher SRP concentration.

The content of the manure in batch 3 may also be due to its sampling location. It appeared to be less homogeneous than batches 1 and 2. For instance, there was a large amount of hay in batch 3, making it more difficult to gather homogeneous samples for SRP analysis. This may explain the large error bars in batch 3 and the relatively lower error bars in batches 1 and 2. Few statistically significant conclusions could be made in batch 3 as to the effect of lime pretreatment on SRP concentrations, operating under the various conditions.

When comparing the results of this experiment to the results of others, SRP concentrations in dairy cow manure were discovered to be within the same two magnitudes of order. The majority of the sources found a slightly higher SRP concentration than those found in this experiment. In order to compare the values as

presented in table 4.1, a number of assumptions were made, and these assumptions may account for these variations.

Table 4.1. SRP concentrations (μg P/g DW) in dairy cow manure based on the source.

Source	SRP Concentrations (μg P/g DW)
Virginia Technical Institute 1997[1]	3256.73[7], 3172.14[8]
Hart, Gangwer, and Marx 1997[1,2]	3265.44
Morse et al. 1992, 3048[1,3]	374.98[9], 485.62[10], 721.68[11]
Barnett 1994, 147[4]	5877.60
Brintrup et al. 1992, 31	6417.91[12], 8208.96[13]
Dou et al. 2000, 510[5]	1920.00
Dou et al. 2003, 3792[6]	4240.00
Dou et al. 2002, 2058	2910.00[14], 7130.00[15], 10460.00[16]
Current Experiment	938.74[17], 1104.22[18], 1513.07[19]

[1]TP concentrations were supplied; SRP concentrations were calculated by assuming 44.41% TP is SRP (This number reflects an average of 63.23% (Barnett 1994, 140), 45.40% (Dou et al. 2003, 3792), 40.45% and 28.57% (Ebeling et al. 2002, 285))
[2]66% of 48 lb dry matter is digested
[3]Assume 100 lbs. feces/day (Hart, Gangwer, and Marx 1997)
[4]Inorganic P concentrations, assumed majority is SRP
[5]Extraction time of 2h (H_2O)
[6]4.4 g P/kg DM average intake
[7]0.11 lbs. P/15 lbs. DM
[8]0.15 lbs. P/21 lbs. DM
[9]0.30% P in diet
[10]0.41% P in diet
[11] 0.56% P in diet
[12]86 g/day P intake
[13]73 g/day P intake
[14]3.41 g/kg DM P in diet
[15]5.1 g/kg DM P in diet
[16]6.7 g/kg DM P in diet
[17]Batch 1 before pretreatment
[18]Batch 2 before pretreatment
[19]Batch 3 before pretreatment

One assumption made in the SRP calculation was that SRP accounts for 44.41 percent TP. This number was determined by averaging the following percentages, 63.23 (Barnett 1994, 140), 45.40 (Dou et al. 2003, 3792), 40.45, and 28.57 (Ebeling et al. 2002, 285). In addition, water-soluble phosphorus and inorganic phosphorus were assumed to be equivalent to SRP. The SRP concentrations, as a result of the TP and inorganic

phosphorus assumptions, found in Morse et al. (1992, 3048) and Dou et al (2000, 510), are the most similar to the SRP concentrations before pretreatment in the current experiment.

Effects of Lime Pretreatment and Fermentation on SRP

With the addition of limestone in the fermentation process, fermentation was expected to further reduce SRP concentrations found in pretreated manure. In batch 1, the fermentation process operated at different temperatures, while in batch 2, fermentation varied based on the occurrence of pretreatment and the addition of bromoform. There was a difference in SRP concentrations discovered in only batch 2 based upon pretreatment, limestone, and bromoform variations.

Batch 1

The higher fermentation temperature of series "a" bottles (60ºC) and the lower temperature of series "b" bottles (40ºC) appeared to have the same affect on SRP concentrations. Both series significantly ($p < 0.01$) reduced SRP concentrations; however, the temperature variation did not produce a significant difference in SRP concentrations after fermentation.

Limestone and bromoform were added to both series to neutralize carboxylic acids (Aiello-Mazzarri, Agbogbo, and Holtzapple 2006, 47) and to inhibit "methane-forming bacteria" (Gerardi 2003, 17), respectively. Both limestone and bromoform were added on a need basis, thus differences in each bottle were likely to occur. There was not, however, a significant ($p < 0.01$) difference in the addition of bromoform as there

was for limestone among series “a” and series “b” bottles. SRP concentrations did not appear to be affected by this factor after fermentation.

Batch 2

As was seen in batch 1, the pretreated and fermented cow manure in batch 2 did not have a significant effect on the SRP concentration in the pretreated cow manure filtrate. Fermentation alone had a significant ($p < 0.01$) effect on the SRP concentration found in the cow manure filtrate, but not to the extent of pretreatment and fermentation. The reason for a decrease in SRP concentrations in the fermentation process is likely to be due to the addition of limestone. It is assumed that with the addition of limestone, the same process of phosphorus precipitation occurred as did with the addition of lime. However, instead of the production of hydroxyapatite, perhaps limestone binds to phosphorus to produce another form of calcium phosphate, such as calcium hydrogen phosphate ($CaHPO_4$). While limestone additions in the fermentation process were successful at reducing SRP concentrations in nonpretreated cow manure, limestone additions in pretreated cow manure were not.

Bromoform additions in batch 2 had a significant ($p < 0.01$) effect on SRP concentrations. The acidogenic bottles had higher levels of SRP than the methanogenic bottles in both the pretreated manure and the pretreated and fermented manure. Bromoform may have reduced lime and/or limestone’s ability to precipitate soluble forms of phosphorus into insoluble forms by allowing for the accumulation of acids, thus lowering the pH of the acidogenic bottles. At a lower pH, calcium phosphates are more soluble (van Loon and Duffy 2000, 296), which may explain why SRP concentrations are higher in the acidogenic bottles.

Batches 1 and 2

Fermentation did not further reduce SRP concentrations measured in pretreated cow manure in batch 2, but in batch 1 there was a reduction in SRP concentrations after fermentation. In batch 1, SRP was not affected by a temperature difference. In batch 2, the methanogenic bottles had lower SRP concentrations, which is likely due to the lack of bromoform. The operating conditions in batch 2 that were most successful at reducing SRP concentrations were pretreatment and fermentation without CH_4 inhibition. While fermentation alone in batch 2 exhibited a reduction in SRP concentrations in cow manure, pretreated cow manure facilitated a further reduction. Pretreatment appears to be beneficial in that it not only speeds up the conversion of biomass to carboxylic acids, but it also is more successful at precipitating soluble forms of phosphorus into insoluble forms than fermentation alone.

Effects of Pretreatment and Fermentation on Dry Weight, Ash Content, and Volatile Solids

The trends in dry weight, ash content, and volatile solids were easier to see in batches 1 and 2. After pretreatment, the dry weight and ash content were expected to decrease and volatile solids were expected to remain constant. After fermentation, the dry weight and ash content should further increase while volatile solids should decrease. Variations of these hypotheses were seen in batches 1, 2, and 3.

Batch 1

As the steps in the MixAlco process progressed, the dry weight and ash content significantly ($p < 0.01$) increased in batch 1. The volatile solids remained relatively constant until an increase occurred after the fermentation process. The series “a” bottles

had a significantly ($p < 0.05$) higher dry weight and ash content than the series "b" bottles, but there was not a noticeable difference in volatile solids.

Batch 2

After the pretreatment step, the dry weight and ash content remained constant while the volatile solids decreased. A significant ($p < 0.05$) increase in dry weight and ash content of pretreated and nonpretreated manure followed fermentation. Volatile solids remained constant after fermentation in both pretreated and nonpretreated cow manure. In fermentation, there was a significant ($p < 0.05, 0.01$) difference in ash content and volatile solids as a result of pretreatment. The pretreated and fermented bottles measured a lower volatile solid content than the fermented bottles.

Batch 3

It is difficult to determine any real trends in dry weight, ash content, and volatile solids in batch 3. However, the dry weight of the bottles pretreated at a higher temperature (100ºC) generally increased after pretreatment while the dry weight of the bottles pretreated at a lower temperature (40ºC) generally decreased. The ash content tended to increase with an increase in dry weight and decrease with a decrease in dry weight. The volatile solids generally remained constant after pretreatment. Some of the ANOVA results indicated a significant ($p < 0.05, 0.01$) difference in dry weight, ash content, and volatile solids after pretreatment (see tables B.21-B.23).

Batches 1, 2, and 3

As was seen in batch 1 and at the higher temperature of batch 3, an increase in dry weight after pretreatment was expected due to the addition of lime in the pretreatment

phase. The pretreatment procedures in batch 1 were carried out exactly as they were in batch 2. Perhaps batch 2 did not see an increase in dry weight because the pretreated manure was not thoroughly mixed. Samples gathered from the top would have a lower dry weight than if the sample had been gathered from the bottom.

Batch 3 was a complicated experiment. The difficulty in detecting trends may have been due to the small sample sizes. Two samples from each bottle were gathered to determine the dry weight, ash content, and volatile solids, and there were only 2 aerated and 1 nonaerated bottles at each temperature. The small sample sizes involved could be why the results were inconsistent. Disparities in the results of batch 3 may also be blamed on the loss of water through the loosely sealed lids, or the hourly or daily replenishment of water in the aerated bottles. The difference in temperature may account for the variations seen in the dry weight due to greater losses in moisture at a higher temperature.

After pretreatment, the ash content was expected to increase while the volatile solids were expected to remain constant as a result of limestone additions. Lime is a solid and should thus increase the dry weight of pretreated manure, but should not have any affect on the volatile solids, because after burning, lime ashes and does not volatilize. Variations of these expectations in batches 2 and 3 resulted, and may be due to the above explanations. Batch 1, however, appeared to mirror these expectations.

After fermentation, the dry weight and ash content were expected to increase, while the volatile solids were expected to decrease. In fermentation, limestone was added which likely increased the dry weight and ash content of batches 1 and 2. A significantly ($p < 0.01$) larger amount of limestone was added to the series “a” bottles

than to the series “b” bottles possibly explaining the larger dry weight and ash content in the series “a” bottles.

The volatile solids should have decreased by the end of fermentation because the gases that formed in the bottles throughout the process were released daily. A reduction in volatile solids also helps assess the success of anaerobic digestion (Callaghan et al. 1999, 117), by evaluating the production of biogas (CO_2 and CH_4). Callaghan et al. (1999, 120) found that the volatile solids reduced by 31.1 and 51.8 percent when cattle slurry was used as a substrate. Ghaly (1996, 69) also experienced a reduction in volatile solids at the end of anaerobic digestion.

The experiments performed by Callaghan et al. (1999, 117-122) and Ghaly (1996, 61-72) focused on the production of methane in anaerobic fermentation, which would result in a loss of volatile solids. The majority of the conditions in the current experiment focused on acid production rather than CH_4 production. Thus, the majority of the products were not released as a gas, but were contained in the liquid form. This may explain why there was not a noticeable reduction after fermentation in batches 1 and 2. Differences in methods may have attributed to the discrepancy in volatile solids, because a reduction was neither seen in the methanogenic bottles despite CH_4 production.

Effects of Temperature, Pretreatment, and Bromoform on Acid Production

Temperature (Batch 1)

Temperature did not have a significant effect on total acid production; however, temperature did have a significant ($p < 0.01$) effect on acetic acid (C2) production in the fermentation process of batch 1. An increase in temperature from 40ºC to 60ºC denotes

an increase in acetic acid production. At higher temperatures, the "acid-forming bacteria" may have grown faster (Gerardi 2003, 17, 28), which may explain this result.

Despite the temperature differences in batch 1, acetic acid represents the largest fraction of the total acids, but an even higher fraction of acetic acid exists in the series "a" bottles. In an experiment performed by Ghaly (1996, 66), acetic acid also represented the highest concentration of volatile acids produced during the anaerobic digestion of dairy manure. Many acids are produced in the "acid-forming stage," but acetate is considered to be the most important (Gerardi 2003, 55). The production of acids in anaerobic digestion is a result of the degradation of the soluble compounds that formed in the hydrolysis phase of digestion (Gerardi 2003, 54-56).

Pretreatment (Batch 2)

Pretreatment had a significant ($p < 0.05$) effect on total acid production but not on acetic acid production in the fermentation process of batch 2. In the bottles that were pretreated with lime, the total acid production dramatically increased at the beginning of the experiment. The total acid production in the nonpretreated bottles also increased, but at slower rates. Lime pretreatment improved "the enzymatic hydrolysis of corn stover" (Kaar and Holtzapple 2000, 189), which in turn allowed for the production of acids. As seen in this experiment, lime pretreatment enhanced the production of acids, which may be explained by an improvement in the enzymatic hydrolysis of cow manure.

Bromoform (Batch 2)

Bromoform had a significant ($p < 0.01$) effect on total acid production and acetic acid production in the fermentation process of batch 2. According to Figure 3.11, the

total acid concentration curves were similar for the acidogenic bottles and for the methanogenic bottles. The differences are that for the acidogenic bottles, total acid production continued to grow during the 30-day experiment, while at day 6 and 12, total acid production began to decline in the methanogenic bottles. This is clearly indicative of the affect that bromoform had on acid production. The purpose of bromoform is to inhibit CH_4 (Holtzapple et al. 1999, 613), which kills off the "methane-forming bacteria", allowing for the accumulation of acids (Gerardi 2003, 17,99). Without inhibiting the "methane-forming bacteria," the acids can be converted into biogas, such as CO_2 and CH_4 (Gerardi 2003, 17, 7).

Batches 1 and 2

The success of this experiment was based upon the total volume of acid produced at day 30. In batch 1, the series "a" and the series "b" bottles produced more or less the same volume of acid. In batch 2, the pretreated acidogenic bottles produced the highest volume of acid. But, in comparison to other studies, acid production in this experiment was relatively low. Total acid production reached a high of approximately 10 g/L, whereas Ross and Holtzapple (2001, fig. 6) achieved production as high as approximately 30 g/L.

Differences in acid production may be due to the source of biomass used in the experiment. The substrate used in this experiment entirely consisted of dairy cow manure, while the substrate in the experiment conducted by Ross and Holtzapple (2001, 116) only partially consisted of manure. Cow manure as the sole source of biomass may have exceeded the nitrogen to carbon ratio that is required to make acids. Acid

production in the current experiment did not achieve concentrations as high as 30 g/L, which was likely due to the high levels of nitrogen present in the manure.

Any further discrepancy in the data may be due to the storage of the liquid samples in the freezer until analysis at Texas A&M could occur. The GC at Texas A&M was needed because of the contamination that occurred in the capillary column in the lab where this experiment took place. Due to this contamination, all analyzed liquid samples were deemed unreliable, including those from the first 6 days of batch 1. This is the reason the analysis of the total acid production began on day 7, and does not include day 1 or 6, as seen in batch 2.

Other sources of error may result in the assumption of normality. The bottles that were chosen for liquid analysis on specific days throughout the experiment were assumed to be normal. Bottles were chosen based upon the ability to control CH_4 (acidogenic bottles), the consistent productions of CO_2 (acidogenic and methanogenic bottles) and CH_4 (methanogenic bottles), and remaining near neutral in pH (acidogenic and methanogenic bottles) throughout the 30-day experiment.

There were only a few abnormal samples in batches 1 and 2, but had these samples been more similar to the other bottles operating at the same fermentation conditions, the results may have slightly differed. For instance, the total and acetic acid concentrations of bottle 2P in batch 2 was much lower than bottles 1P and 3P and looked more similar to bottles 1-3. Had bottle 2P been more similar to bottles 1P and 3P, bromoform may not have produced a significant ($p < 0.01$) difference in the acid concentrations between the acidogenic and methanogenic bottles of batch 2.

Effects of Temperature, Pretreatment, and Bromoform on Gas Production

Temperature (Batch 1)

Temperature had a significant ($p < 0.05$) effect on CO_2 production in batch 1. The series "a" bottles operating at a higher temperature produced more CO_2 than the series "b" bottles operating at a lower temperature. Carbon dioxide production also appeared to have increased and peaked earlier in the series "a" bottles. Both phenomenon could be explained by the enhanced growth of the bacteria present in the digester whose job is to break down the organic compounds into volatile acids and gases, such as CO_2 (Gerardi 2003, 54).

Pretreatment (Batch 2)

Pretreatment did not have a significant effect on either CO_2 or CH_4 production in batch 2. Pretreatment was expected to decrease CO_2 and CH_4 production in the acidogenic bottles to favor acid accumulation, and increase CO_2 and CH_4 production in the methanogenic bottles. There was some evidence of this in the methanogenic bottles, but not in the acidogenic bottles. Lime pretreatment appears to have aided more in the production of acids than in the production of gases.

Bromoform (Batch 2)

Bromoform had a significant ($p < 0.01, 0.05$) effect on both CO_2 and CH_4 production in batch 2. The addition of bromoform suppressed CH_4 production and decreased CO_2 production. The "methane-producing bacteria" (Gerardi 2003, 17) were less likely to survive in the methanogenic bottles, thus favoring an accumulation of acids.

In the acidogenic bottles the depressed CO_2 volume may be explained by the high conversion of organic compounds into acetic acid (Gerardi 2003, 15, 55).

In the methanogenic bottles, there appears to be an exponential increase in the production of CH_4 at day 9 or 10. Svendsen and Blackburn (1986, 61), who experimented with swine manure and hay mixtures, also noticed an "exponential increase in the methane production rate," but at about day 15 rather than day 9. They attributed this increase in production to the simultaneous growth in methanogenic bacteria (Svendsen and Blackburn 1986). Despite the differences in substrates, this is a plausible explanation as to why CH_4 production increased in the fermentation of batch 2. By phase III, day 47-62, CH_4 production was at a maximum and was followed by a decrease (Svendsen and Blackburn 1986, 62). Methane production in the methanogenic bottles began to level off by day 28; however, its decline cannot be seen in this 30-day period.

Batches 1 and 2

Limestone was added in both batch 1 and 2, and may have also affected gas production. The addition of limestone in the fermentation process aided in the neutralization of carboxylic acids ((Aiello-Mazzarri, Agbogbo, and Holtzapple 2006, 47) so that bacteria may thrive to help in the conversion of biomass. In the neutralization process, abiotic CO_2 is released as a result, while biotic CO_2 is considered to be a direct product of fermentation (Thanakoses, Mostafa, and Holtzapple 2003, 531). The abiotic and biotic volumes may be calculated based upon the stoichiometry of the neutralization which results in: "1 mol of abiotic CO_2 is produced for every 2 mol of acid produced" (Thanakoses, Mostafa, and Holtzapple 2003). By day 30, there was significantly

($p < 0.01$) more limestone added to the series "a" bottles of batch 1, which, in addition to temperature, may be a reason as to why CO_2 production was greater.

Sources of error may have originated from the analysis of gases by the GC. Meaning, that the gas chromatographs produced by the GC did not always show well separated curves. This could be attributed to human error, such as the result of a bad injection or the need to inject the standard. No sense could be made of these types of chromatograms, thus they were disregarded. Gas production may not be completely accurate as a result of the data gaps, but at least the remaining chromatograms were able to partially indicate what was occurring.

In addition, the GC was not working properly for the first 9 days of batch 2. The gas samples produced during this time had to remain stored in the syringes used to sample the gas. The syringe needles were stored in rubber stoppers until analysis could occur after day 9. Due to the large amount of samples needing to be analyzed, analysis took more than 1 day. According to figures 3.12 to 3.15, gas production was rather low for the first 9 days. This could be due to the loss of some gas from the syringe as a result of its storage. In addition, CH_4 was more difficult to control in batch 2 than in batch 1 as a result of the inability to see any CH_4 production. The volume and frequency of bromoform additions were based upon the results of batch 1. Had the GC been working properly at the start of the experiment, CH_4 inhibition may have been just as successful in batch 2, and gas production over the first 9 days of fermentation would have been a more realistic representation.

Effect of Fermentation Conditions on pH

Temperature (Batch 1)

Temperature did not have a significant effect on the pH of batch 1. This may be due to the insignificant differences in total acid production between the two series. A higher concentration of acids would ultimately decrease the pH. While the difference in pH is not significant, the pH of the series "b" bottles appears to be slightly higher than the pH of the series "a" bottles. This may be indicative of the higher proportion of acetic acid in series "a" bottles, which has a lower pKa than the longer chain acids (Weast and Astle 1978, D202-D203).

Bromoform (Batch 2)

Bromoform had a significant ($p < 0.01$) difference on the pH of batch 2. An accumulation of acids was able to occur in the acidogenic bottles, which is the main reason these bottles had a lower pH by day 30 of the fermentation process. The pH of the acidogenic bottles remained relatively stable throughout the 30 days, but the pH of the methanogenic bottles increased around day 12. An increase in pH shows the consumption of the volatile acids by the "methane-forming bacteria" (Gerardi 2003, 17, 99), while the stable pH may indicate the simultaneous neutralization and accumulation of carboxylic acids.

Batches 1 and 2

The average pH values in batches 1 and 2 varied from about 6.15 to 7.03 and 5.71 to 7.46, respectively. The pH in batch fermentations in municipal solid waste performed by Aiello-Mazzarri, Agbogbo, and Holtzapple (2006, 51) ranged from 5.4 to 6.5. "Acid-

forming bacteria" prefer a pH below 5.0, while "methane-forming bacteria" prefer a pH at or above 6.20, but not exceeding a pH of 8.00 (Gerardi 2003, 99). Better acid and methane production could have resulted if the pH of the bottles were more closely associated to these preferred values.

Conclusion

The analysis of SRP and pretreatment and batch fermentation parameters in the MixAlco process produced some rather interesting results that may be applied to the Lake Waco watershed. The results of TP analysis were not discussed in this project due to difficulties in obtaining quality data, but the results are presented in Appendix A.

Lime Pretreatment

With the intent of increasing biomass digestibility, lime pretreatment was also successful at decreasing SRP concentrations measured in the dairy cow manure filtrate. The various pretreatment conditions indicated that pretreatment for 2 h at 100°C, with a lime loading of 0.10 g/g DW of manure, and with aeration were the most successful at reducing SRP concentrations.

Fermentation

Fermentation also decreased SRP concentrations in the pretreated manure filtrate, but fermentation alone did not have the same effect on SRP concentrations as pretreatment and fermentation. Limestone and bromoform additions also affected the precipitation of soluble forms of phosphorus into insoluble forms. Limestone was probably the reason for a reduction in SRP, while bromoform likely reduced limestone's precipitation ability.

The Lake Waco Watershed

The MixAlco process appears to successfully reduce SRP concentrations measured in dairy cow manure. Phosphorus is most often the nutrient limiting algal growth in freshwater systems, thus a decrease in the soluble fraction of phosphorus available for algal uptake may in turn decrease the effects of eutrophication. This may have a positive impact on the Lake Waco watershed because dairy manure used in agricultural operations is a major cause of nonpoint source pollution. By using dairy cow manure as a source of biomass, the manure that would otherwise be subject to runoff would be removed. If the remaining steps of the MixAlco process mirror the first 2, the waste would have a lower concentration of SRP than the input. As a result, Central Texas could benefit in terms of its water quality, as well as from an alternate energy source.

Dairy Manure

In terms of evaluating the success of dairy cow manure as a source of biomass in the MixAlco process, the results of this study indicate that manure as the sole source many not be as successful as if it were mixed with other substrates. When manure was used as the sole source, acid production was approximately 3 times lower than it was in an experiment conducted by Ross and Holtzapple (2001, fig. 6), who used mixtures of manure, municipal solid wastes, and sewage sludge. However, pretreatment enhanced the digestibility of cow manure and increased the production of acids in both the acidogenic and methanogenic bottles.

Further Studies

Further studies should concentrate on ways to improve the production of acids, which would make the MixAlco process economically viable. Focus should also be placed on improving the analysis performed in this experiment, as well as expanding it to include the remaining steps of the MixAlco process. Analyses should not only include phosphorus measurement, but nitrogen concentrations should also be considered due to the growing importance of colimitation.

APPENDICES

APPENDIX A

Data Tables

Table A.1. SRP concentrations (µg P/g DW) in dairy cow manure, pretreated dairy cow manure (2h 100ºC), and fermented dairy cow manure (a=60ºC, b=40ºC) (batch 1).

SRP in dairy cow manure			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or – 1)
1	817.64		
2	1032.76		
3	987.94		
4	852.91		
5	966.04		
6	916.52		
7	925.65		
8	1010.43	938.74	75.44
SRP in pretreated dairy cow manure (2h 100°C)			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or – 1)
1	213.11		
2	198.35		
3	297.35		
4	223.35		
5	187.79		
6	183.28		
7	214.75		
8	398.76	239.59	73.49
SRP in fermented dairy cow manure (a=60°C, b=40°C)			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or – 1)
1a	55.54		
2a	73.63		
3a	156.83		
4a	79.17		
5a	106.59		
6a	85.08	92.81	35.47
1b	137.92		
2b	118.42		
3b	121.23		
4b	37.22		
5b	102.03		
6b	90.97	101.30	35.34

Table A.2. TP concentrations (µg P/g DW) in dairy cow manure, pretreated dairy cow manure (2h 100ºC), and fermented dairy cow manure (a=60ºC, b=40ºC) (batch 1).

TP in dairy cow manure			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or – 1)
1			
2	4483.92		
3	4189.72		
4	3539.93		
5	4072.29		
6	4945.35		
7	4185.64		
8	3819.23	4176.58	452.70
TP in pretreated dairy cow manure (2h 100°C)			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or – 1)
1	4701.30		
2	6670.43		
3	7004.32		
4	5769.68		
5	6452.67		
6	6238.85		
7	6119.72		
8	6451.46	6176.05	700.00
TP in fermented dairy cow manure (a=60°C, b=40°C)			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or – 1)
1a	3682.32		
2a	3919.00		
3a	3546.93		
4a	3617.57		
5a	3712.31		
6a	3130.38	3601.42	262.60
1b	3212.15		
2b	3743.76		
3b	3455.73		
4b	3359.49		
5b	3395.59		
6b	3778.17	3490.82	224.39

Table A.3. SRP concentrations (µg P/g DW) in dairy cow manure, pretreated dairy cow manure (2h 100ºC), and fermented dairy cow manure (40ºC, 1-3=no pretreatment, no bromoform, 1B-3B=no pretreatment, bromoform, 1P-3P=pretreatment, no bromoform, 1PB-3PB=pretreatment, bromoform) (batch 2).

SRP in dairy cow manure			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or - 1)
1	912.57		
2	918.07		
3	1455.20		
4	697.03		
5	933.36		
6	1110.32		
7	1101.91		
8	1705.32	1104.22	327.53
SRP in pretreated dairy cow manure (2h 100°C)			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or - 1)
1	97.48		
2	56.15		
3	89.16		
4	341.99		
5	111.35		
6	560.21		
7	135.38		
8	141.21	191.62	172.51
SRP in fermented dairy cow manure (40ºC, B=bromoform, P=pretreatment)			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or - 1)
1	203.21		
2	158.57		
3	197.62	186.47	24.32
1B	366.50		
2B	458.51		
3B	466.70	430.57	55.63
1P	83.69		
2P	80.01		
3P	53.38	72.36	16.54
1PB	117.37		
2PB	128.83		
3PB	129.17	125.12	6.71

Table A.4. TP concentrations (µg P/g DW) in dairy cow manure, pretreated dairy cow manure (2h 100ºC), and fermented dairy cow manure (40ºC, 1-3=no pretreatment, no bromoform, 1B-3B=no pretreatment, bromoform, 1P-3P=pretreatment, no bromoform, 1PB-3PB=pretreatment, bromoform) (batch 2).

TP in dairy cow manure			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or - 1)
1	6004.14		
2	6931.97		
3	8352.69		
4	7975.35		
5	5946.83		
6	9872.74		
7	10480.28		
8	6970.49	7816.81	1686.71
TP in pretreated dairy cow manure (2h 100°C)			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or - 1)
1	822.79		
2	961.97		
3	2179.49		
4	1190.41		
5	1490.26		
6	1490.66		
7	1228.99		
8	1660.54	1378.14	428.66
TP in fermented dairy cow manure (40ºC, B=bromoform, P=pretreatment)			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or - 1)
1	6154.30		
2	6831.20		
3	7253.15	6746.24	554.29
1B	5401.37		
2B	7100.92		
3B	8823.71	7108.67	1711.18
1P	9180.59		
2P	8228.10		
3P	7825.92	8411.54	1695.71
1PB	6124.88		
2PB	7272.08		
3PB	7231.92	6876.30	651.06

Table A.5. SRP concentrations (µg P/g DW) in dairy cow manure and pretreated dairy cow manure (2h 100ºC, 7d 40ºC) (batch 3).

SRP in dairy cow manure			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or – 1)
1	1458.11		
2	2348.67		
3	2410.78		
4	2534.38		
5	1268.76		
6	786.52		
7	683.59		
8	613.74	1513.07	813.69
SRP in pretreated dairy cow manure (2h 100°C)			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or – 1)
1a air 10% lime	636.13		
1b air 10% lime	325.63		
2a air 10% lime	320.03		
2b air 10% lime	211.92	373.43	182.78
1a no air 10% lime	869.37		
1b no air 10% lime	564.98	717.18	215.24
1a air 5% lime	711.14		
1b air 5% lime	481.02		
2a air 5% lime	728.29		
2b air 5% lime	456.62	594.27	145.37
1a no air 5% lime	996.54		
1b no air 5% lime	778.33	887.43	154.29
SRP in pretreated dairy cow manure (7d 40°C)			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or – 1)
1a air 10% lime	1052.38		
1b air 10% lime	650.50		
2a air 10% lime	987.98		
2b air 10% lime	514.92	801.44	259.90
1a no air 10% lime	1431.31		
1b no air 10% lime	964.93	1198.12	329.78
1a air 5% lime	1193.83		
1b air 5% lime	765.77		
2a air 5% lime	1234.98		
2b air 5% lime	1555.09	1187.42	324.18
1a no air 5% lime	1696.16		
1b no air 5% lime	1515.05	1605.61	128.06

Table A.6. TP concentrations (µg P/g DW) in dairy cow manure and pretreated dairy cow manure (2h 100ºC, 7d 40ºC) (batch 3).

TP in dairy cow manure			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or – 1)
1	28242.11		
2	32665.93		
3	33254.21		
4	32791.83		
5	35570.47		
6	33630.86		
7	34926.53		
8	36475.27	33444.65	2509.12
TP in pretreated dairy cow manure (2h 100°C)			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or – 1)
1a air 10% lime	9891.15		
1b air 10% lime	10898.76		
2a air 10% lime	8027.03		
2b air 10% lime	8842.58	9414.88	1249.31
1a no air 10% lime	10663.36		
1b no air 10% lime	7644.20	9153.78	2134.87
1a air 5% lime	8919.67		
1b air 5% lime	7933.00		
2a air 5% lime	8994.11		
2b air 5% lime	9205.07	8762.97	566.36
1a no air 5% lime	8249.03		
1b no air 5% lime	8061.75	8155.39	132.43
TP in pretreated dairy cow manure (7d 40°C)			
Sample	µg P/g DW	Average µg P/g DW	SD (+ or – 1)
1a air 10% lime	7577.10		
1b air 10% lime	8828.55		
2a air 10% lime	8644.91		
2b air 10% lime	8316.36	8341.73	552.03
1a no air 10% lime	11594.95		
1b no air 10% lime	16638.36	14116.66	3566.23
1a air 5% lime	8620.74		
1b air 5% lime	8568.20		
2a air 5% lime	9077.61		
2b air 5% lime	9603.95	8967.63	481.97
1a no air 5% lime	8184.57		
1b no air 5% lime	8863.57	8524.07	480.13

Table A.7. Total acid concentrations (g/L) in fermenting bottles 6a (60ºC), 1b (40ºC), 1 (40ºC, no pretreatment, no bromoform), 3B (40ºC, no pretreatment, bromoform), 2P (40ºC, pretreatment, no bromoform), 2PB (40ºC, pretreatment, bromoform) (batches 1 and 2). Total acid concentrations obtained day 7 for batch 1, and day 6 for batch 2.

	Sample					
Day	6a	1b	1	3B	2P	2PB
1			2.35	2.37	3.20	2.85
6/7	4.07	4.41	3.59	3.84	7.88	6.25
12	6.72	6.34	3.46	4.94	8.41	7.84
18	6.58	6.96	2.22	5.40	5.30	8.46
24	5.57	5.26	1.74	5.75	3.88	8.50
30	7.25	6.76	1.07	6.04	2.13	8.28

Note: Total acid concentrations obtained day 7 is for batch 1 and total acid concentrations obtained day 6 is for batch 2

Table A.8. Total acid and C2 concentrations (g/L) in fermenting bottles 1a-6a (60ºC) and 1b-6b (40ºC) at day 30 (batch 1), and 1-3 (40ºC, no pretreatment, no bromoform), 1B-3B (40ºC, no pretreatment, bromoform), 1P-3P (40ºC, pretreatment, no bromoform), and 1PB-3PB (40ºC, pretreatment, bromoform) (batch 2).

Sample	Total	C2	Average (Total/C2)	Sample	Total	C2	Average (Total/C2)
1a	4.51	3.79		1	1.07	0	
2a	6.18	5.48		2	1.31	0	
3a	6.95	6.31		3	1.91	0	1.43 / 0.00
4a	6.78	6.07		1B	5.53	2.72	
5a	7.42	6.66		2B	6.85	3.37	
6a	7.25	6.64	6.52 / 5.83	3B	6.04	3.15	6.14 / 3.08
1b	6.76	3.85		1P	6.60	2.76	
2b	7.36	4.04		2P	2.13	0.12	
3b	4.44	3.16		3P	7.85	3.74	5.53 / 2.21
4b	7.54	3.95		1PB	7.50	3.23	
5b	4.93	2.77		2PB	8.28	3.53	
6b	8.38	5.03	6.57 / 3.80	3PB	3.32	3.32	7.92 / 3.36

Table A.9. CO_2 volumes (mL) produced in fermenting bottles 1a-6a (60ºC) (batch 1).

	Sample					
Day	1a	2a	3a	4a	5a	6a
1		337.13	167.24	20.66		29.57
2	83.21	2218.54	1961.30	1456.57	7299.14	7151.40
3	10253.00	7631.52	8905.04	15295.63		10610.05
4	239.07	292.78	537.72			371.91
5	4086.65	4874.95	6672.15	3783.30	3789.66	4301.16
6	2010.76	1763.46	1907.11	2599.45	2910.79	2496.65
7	1881.12	1922.63	6213.06	1815.68	2268.42	5699.84
8	1930.77	1633.99	3537.16	2274.43	2426.48	1697.31
9	5542.39	1558.35	2360.07	3575.24		
10	944.77	1677.77	3260.69	2590.56	2331.48	907.08
11	1376.09	1345.87	2556.10	1608.85	1876.41	1001.11
12	1640.73	1233.76	2020.82	2188.46	1333.44	1084.28
13	1426.24	1195.99	1420.15			873.68
14			1912.47	1088.17	1712.09	
15	669.41	1091.51	755.09	1764.58	563.30	438.94
16	436.31	224.73	750.81	653.35	549.99	437.10
17	343.74	406.08	580.15	481.40		374.10
18	500.92	372.67	515.03	330.79	311.70	202.59
19	260.96	420.50	617.73		242.41	443.42
20	260.92	350.02	286.60	823.18	235.62	347.09
21	295.79	353.86	359.14	333.51	303.87	5.61
22	127.44	171.41		216.37		170.05
23	287.45	140.06	316.33	200.19	179.34	167.02
24	553.53	28.07	269.70	150.41	128.60	97.89
25	270.51	3.70	243.23	243.65		
26	253.92		369.85	274.25	154.15	290.22
27	464.97	331.24	278.02	288.68	116.61	287.83
28		131.99	380.01	199.72	241.54	177.08
29		188.32	346.29	141.70	138.46	209.26
30	138.35	47.96	275.14	166.55	98.75	152.83

Note: The data are based on individual volumes rather than cumulative volumes.

Table A.10. CO_2 volumes (mL) produced in fermenting bottles 1b-6b (40ºC) (batch 1).

	Sample					
Day	1b	2b	3b	4b	5b	6b
1	246.39	151.38	133.92	132.10	182.10	183.37
2	1842.94	2197.23	1106.45		1765.43	
3	3555.00	3564.08	2230.09	2325.69		3131.07
4		3.08		21.37	311.76	30.40
5	2961.69	3166.92	1626.54	2500.99	1777.33	3317.89
6	4644.41	1191.15	926.26	1470.07		1799.96
7	4930.95		1412.63			2870.00
8	1438.95	1039.46	6387.73	844.26	1180.04	855.70
9	2475.59	8924.63				
10	1376.36	1448.94	2121.20	478.99	957.11	773.75
11	1760.74	969.76	1208.92	935.64	687.66	1211.09
12	678.17	876.57	1314.00	895.30	709.76	
13	1148.66	608.58	3575.44	1555.31	775.45	664.70
14		794.04	1680.34	1687.91	1167.66	978.46
15	718.24	722.17	1897.71	4033.03	2326.88	2205.57
16	561.73	3041.33	1155.88	385.39	1892.37	278.70
17	214.47	729.16	296.39	5.21	571.88	612.45
18	1512.90		566.03	7.94	921.81	449.93
19	1138.22	2887.11	959.23	1384.00	875.12	809.33
20						
21	1427.11		407.84	261.15	378.35	485.65
22	588.20	272.03	347.00	1.47	324.19	238.72
23	592.05	81.71	346.42	61.17	326.15	156.46
24	299.30	230.42	247.28	424.67	306.04	111.20
25						13.47
26		289.56	270.53	92.83	277.04	48.50
27	643.44	588.80	534.94	71.32	285.96	
28	317.46	319.05	267.76	63.22	243.33	200.32
29	214.20	127.18			139.64	105.36
30	307.51	330.84	351.05	777.41	163.38	84.48

Note: The data are based on individual volumes rather than cumulative volumes.

Table A.11. CO_2 volumes (mL) produced in fermenting bottles 1-3 (40ºC, no pretreatment, no bromoform) and 1B-3B (40ºC, no pretreatment, bromoform) (batch 2).

	Sample					
Day	1	2	3	1B	2B	3B
1	51.57	70.32	40.67		45.41	5.97
2	6.27	40.55	118.24	0.14	126.67	
3	44.59	34.84	65.47	31.73		78.55
4	46.89	37.86	85.28	31.24	37.88	
5	85.73	89.18	160.44	36.96	54.59	62.26
6	197.73	144.70	198.52	155.82	66.49	23.87
7	239.28		256.05	74.55	56.91	91.55
8			383.13	159.19		157.38
9	964.88	384.00	1024.85	269.70	175.54	213.98
10	1483.04	583.57	2117.11		366.25	297.64
11		804.19	2380.30	161.89	250.02	370.78
12		1200.36	1702.03		270.52	219.33
13	1565.54	1135.73	1640.56	161.27		
14	985.54	713.78	960.35	168.09		140.63
15	666.72	520.53		204.91	170.66	168.29
16	768.45	574.46	1167.96		95.44	
17	461.72	552.46	679.11		118.87	100.31
18	782.90	585.50	708.69		17.95	
19	610.67		655.37	77.81		
20	880.32	607.26	958.03	56.81		153.84
21	709.65		511.46	61.21	0	83.99
22	532.34	535.75		67.73	104.55	
23	749.26	660.14	718.46	128.51	65.40	
24	612.40	522.33	668.06	94.42	52.62	
25						103.59
26	760.74	536.60	1406.90	83.65	55.24	52.77
27	619.82	597.05		100.09	63.07	36.52
28	1095.58	772.90	1161.80	77.98		52.00
29	584.58	456.81	851.14	91.73	50.93	32.46
30				57.43	53.00	54.62

Note: The data are based on individual volumes rather than cumulative volumes.

Table A.12. CO_2 volumes (mL) produced in fermenting bottles 1P-3P (40ºC, pretreatment, no bromoform) and 1PB-3PB (40ºC, pretreatment, bromoform) (batch 2).

	Sample					
Day	1P	2P	3P	1PB	2PB	3PB
1	78.26	59.40	26.82	31.95	25.27	38.36
2			61.67	194.63	216.23	143.45
3	150.46	156.60	55.74			
4	213.99	155.22	246.85	109.88	256.25	91.59
5	177.84	269.12	357.70	89.10	170.02	154.78
6	108.83	319.82	346.21	109.47	34.29	
7	490.94	123.46	199.44	348.21		230.13
8	408.85	376.37	270.36	435.15		
9	610.86	441.10	376.77	466.72	403.97	
10	887.95	508.66				
11		571.90	300.73	693.13	524.77	321.35
12	462.56	665.00		377.20		
13					720.25	219.78
14	472.54	1106.85	193.33	170.78	221.89	201.55
15	330.41	879.77		409.08	352.88	194.03
16	722.19	1362.03	264.09	333.50	336.33	
17		992.01	228.29		174.61	162.80
18	84.89				151.03	146.56
19		911.19	131.17		156.94	134.69
20	908.92		275.02	204.28	247.18	137.12
21	541.26	494.76	197.31	76.29	123.79	81.69
22	404.47	528.81	201.98	134.44	83.13	118.40
23	572.80	684.07	295.00	150.65	115.90	228.34
24						
25	510.17	813.50	655.67	143.94	103.57	76.67
26	347.19	1378.30	640.27	51.42	108.02	34.74
27			584.56	131.74	109.75	124.29
28	323.08	1141.47	249.41	7.23	59.36	182.78
29	190.01	408.77	172.56	128.65	183.07	76.59
30	205.24	378.67	247.51	95.51	101.61	78.51

Note: The data are based on individual volumes rather than cumulative volumes.

Table A.13. CH_4 volumes (mL) produced in fermenting bottles 1a-6a (60ºC) (batch 1).

	Sample					
Day	1a	2a	3a	4a	5a	6a
1		0.38	0	0		0
2	0	0	0	0	0	0
3	17465.65	5.20	0	0		0
4	0.25	0	0			0
5	0	0	0	0	0	0
6	7.37	0	0	0	0	0
7	15.07	0.58	0	0	0	0
8	5.70	0	0	0	0	0
9	10.96	0	0	0		
10	1.82	0	0	0	0	0
11	9.57	0	0	0	0	0
12	17.92	0	0	0	0	0
13	24.38	0	0			0
14			0	0	0	
15	11.92	0.71	0	0	0	0
16	10.94	0	0	0	0	0
17	0	4.58	0	0		0
18	12.97	0	0	0	0	0
19	11.65	0	0		0	0
20	0.47	0	0	0	0	0
21	14.07	0.56	0	0	0	0
22	1.77	0		0		0
23	9.50	0	0	0	0	0
24	8.61	0	0	0	0	0
25	2.59	0	0	0		
26	8.20	0		0	0	0
27	15.66	0	0	0	0	0
28		0	0	2.45	0	0
29		0	0	0	0	0
30	1.88	0	0	0	0	0

Note: The data are based on individual volumes rather than cumulative volumes.

Table A.14. CH_4 volumes (mL) produced in fermenting bottles 1b-6b (40ºC) (batch 1).

	Sample					
Day	1b	2b	3b	4b	5b	6b
1	0	0	0	0	0	0
2	0	0	0		0	
3	0	0	0	0		0
4		0		0	6.89	0
5	0	0	0	0	0	0
6	0	0	0	0		0
7	0		0			0
8	0	0	0	0	0	0
9	0	0				
10	0	0	0	0	0	0
11	0	0	0	0	0	0
12	0	0	0	0	0	
13	0	0	0	0	0	0
14		0	0.36	0.60	0	0
15	0	0	0	0	0	0
16	0	0	0	0	0	0
17	0	0	0	0	0	5471.03
18	0		0	0	0	0
19	0	0	0	0	0	0
20						
21	0		0	0	0	0
22	0	0	0	0	0	0
23	0	0	0	0	0	0
24	0	0	0	0	0.078	0
25						0
26		0	0	0	0	0
27	0	0	0	0	0	
28	0	0	0	0	0	0
29	0	0			0	0
30	0	0	0	0	0	0

Note: The data are based on individual volumes rather than cumulative volumes.

Table A.15. CH_4 volumes (mL) produced in fermenting bottles 1-3 (40ºC, no pretreatment, no bromoform) and 1B-3B (40ºC, no pretreatment, bromoform) (batch 2).

	Sample					
Day	1	2	3	1B	2B	3B
1	66.45	1.02	32.40		0	0
2	8.27	19.51	15.89	1.65	1.02	
3	16.46	14.42	43.09	0		0
4	34.65	3.90	0.90	0	0	
5	71.38	103.22	41.88	0	0	0
6	218.83	188.79	168.18	0	0	0
7	238.28		265.08	1.25	0	0
8			685.85	4.86		0
9	936.92	239.85	1359.16	30.55	0.28	0
10	1326.34	480.42	2229.03		0.55	0
11		882.49	4080.01	2.73	0	0
12		1425.63	2288.27		0	0
13	2412.38	1745.43	2293.53	0		
14	1540.32	1413.29	1163.23	3.13		0
15	815.70	918.30		5.26	0	0
16	742.45	590.03	610.82		0.28	
17	330.08	419.30	1003.70		0	0
18	674.15	326.18	876.62		0	
19	680.43		1541.02	4.41		
20	1615.46	479.37	1376.74	3.47		0
21	1300.40		1639.11	11.15	0	0.55
22	516.88	477.38		0	0	
23	0	0	17.28	4.17	0	
24	393.95	429.53	786.73	3.27	0.26	
25						13.11
26	612.99	316.10	13478.40	6.23	0	0
27	462.76	486.87		14.14	1.60	0
28	794.71	615.25	947.36	10.31		0
29	464.28	352.71	736.25	10.91	1.48	0
30				5.40	1.01	0.70

Note: The data are based on individual volumes rather than cumulative volumes.

Table A.16. CH_4 volumes (mL) produced in fermenting bottles 1P-3P (40ºC, pretreatment, no bromoform) and 1PB-3PB (40ºC, pretreatment, bromoform) (batch 2).

	Sample					
Day	1P	2P	3P	1PB	2PB	3PB
1	5.18	0	0	0	0	0
2			0	0	0	0
3	0	0	0			
4	5.61	0	0	0	0	0
5	2.28	1.40	0	0	0	0
6	18.89	8.91	0	0	0	
7	25.10	2.04	0	0		0
8	19.50	46.18	0	0		
9	119.92	187.28	0.83	0	0	
10	197.67	270.83				
11		306.82	0.47	0	0	0.17
12	99.30	620.31		0		
13					1803.12	7.05
14	136.29	3020.19	6.35	0.21	0	0
15	144.37	1980.69	0	0	0	0
16	522.30	2272.85	23.35	1.45	0.51	
17		1399.54	1.60		0	16.73
18	0				7.46	0
19		1029.91	4.88		0	0
20	1412.60		38.29	0.33	0	0
21	566.32	581.23	56.29	1.17	0	0
22	292.55	432.78	48.38	0.65	0	0
23	0	0	169.72	0	112.54	1.62
24						
25	179.28	1294.52	1023.15	13.11	0	0
26	145.61	10898.59	945.70	1.13	0.17	0.23
27			406.43	2.39	0.97	1.01
28	87.56	1882.45	84.21	0.030	0	0
29	93.38	429.16	46.69	0.69	0.89	0.56
30	58.01	296.18	27.12	1.120	0.82	0.61

Note: The data are based on individual volumes rather than cumulative volumes.

Table A.17. pH trends in fermenting bottles 1a-6a (60ºC) (batch 1).

	Sample						
Day	1a	2a	3a	4a	5a	6a	Average
1	6.90	7.00	7.00	6.96	6.98	6.83	6.95
2	6.87	6.66	6.70	6.91	6.35	6.41	6.65
3	6.00	6.17	6.24	6.18	6.15	6.16	6.15
4	6.08	6.19	6.22	6.20	6.28	6.33	6.22
5	6.10	6.26	6.21	6.21	6.14	6.24	6.19
6	6.33	6.31	6.24	6.26	6.12	6.26	6.25
7	6.07	6.21	6.18	6.23	6.08	6.20	6.16
8	6.17	6.30	6.27	6.37	6.28	6.44	6.31
9	6.12	6.26	6.14	6.19	6.12	6.29	6.19
10	6.18	6.27	6.11	6.15	6.20	6.27	6.20
11	6.11	6.23	6.07	6.16	6.21	6.27	6.18
12	6.11	6.22	6.03	6.13	6.13	6.28	6.15
13	6.08	6.28	6.02	6.16	6.13	6.26	6.16
14	6.09	6.28	6.07	6.17	6.16	6.28	6.18
15	6.15	6.41	6.17	6.34	6.27	6.42	6.29
16	6.17	6.37	6.11	6.23	6.21	6.36	6.24
17	6.13	6.34	6.10	6.23	6.18	6.38	6.23
18	6.24	6.45	6.21	6.29	6.25	6.40	6.31
19	6.38	6.15	6.26	6.28	6.23	6.38	6.28
20	6.28	6.41	6.23	6.33	6.29	6.46	6.33
21	6.33	6.45	6.23	6.34	6.35	6.46	6.36
22	6.36	6.50	6.25	6.37	6.35	6.48	6.39
23	6.42	6.50	6.29	6.39	6.43	6.51	6.42
24	6.50	6.49	6.27	6.36	6.38	6.47	6.41
25	6.71	6.59	6.32	6.40	6.38	6.53	6.49
26	6.77	6.54	6.30	6.38	6.36	6.47	6.47
27	6.71	6.51	6.34	6.37	6.39	6.46	6.46
28	6.78	6.54	6.41	6.38	6.41	6.40	6.49
29	6.75	6.54	6.45	6.39	6.38	6.45	6.49
30	6.80	6.59	6.45	6.44	6.41	6.52	6.54

Table A.18. pH trends in fermenting bottles 1b-6b (40ºC) (batch 1).

	Samples						
Day	1b	2b	3b	4b	5b	6b	Average
1	7.02	6.92	7.03	7.09	7.06	7.05	7.03
2	6.68	6.61	6.74	6.71	6.68	6.70	6.69
3	6.27	6.54	6.47	6.38	6.52	6.61	6.47
4	6.42	6.58	6.58	6.41	6.40	6.72	6.52
5	6.54	6.47	6.55	6.44	6.35	6.51	6.48
6	6.42	6.44	6.51	6.43	6.40	6.55	6.46
7	6.64	6.64	6.69	6.57	6.57	6.72	6.64
8	6.61	6.63	6.63	6.60	6.60	6.68	6.63
9	6.52	6.70	6.53	6.72	6.66	6.75	6.65
10	6.62	6.51	6.52	6.62	6.61	6.67	6.59
11	6.68	6.56	6.54	6.54	6.53	6.60	6.58
12	6.66	6.61	6.56	6.60	6.59	6.65	6.61
13	6.72	6.68	6.36	6.71	6.64	6.70	6.64
14	6.61	6.68	6.32	6.36	6.48	6.48	6.49
15	6.67	6.63	6.41	6.47	6.36	6.42	6.49
16	6.63	6.36	6.42	6.43	6.33	6.38	6.43
17	6.67	6.54	6.61	6.63	6.52	6.56	6.60
18	6.31	6.44	6.38	6.43	6.31	6.35	6.37
19	6.57	6.32	6.53	6.60	6.47	6.54	6.51
20	6.33	6.52	6.47	6.59	6.45	6.56	6.49
21	6.42	6.51	6.49	6.62	6.62	6.51	6.60
22	6.48	6.56	6.52	6.52	6.60	6.52	6.60
23	6.57	6.59	6.54	6.54	6.71	6.58	6.62
24	6.43	6.49	6.45	6.45	6.81	6.58	6.57
25	6.55	6.63	6.57	6.57	6.98	6.68	6.68
26	6.61	6.65	6.56	6.56	7.07	6.66	6.71
27	6.59	6.58	6.53	6.53	7.05	6.62	6.71
28	6.55	6.58	6.54	6.54	7.10	6.62	6.70
29	6.74	6.65	6.60	6.60	7.16	6.76	6.65
30	6.52	6.54	6.58	6.58	7.04	6.64	6.76

Table A.19. pH trends in fermenting bottles 1-3 (40ºC, no pretreatment, no bromoform) and bottles 1B-3B (40ºC, no pretreatment, bromoform) (batch 2).

	Sample				Sample			
Day	1	2	3	Average (1-3)	1B	2B	3B	Average (1B-3B)
1	5.78	5.91	5.84	5.84	5.93	5.82	5.87	5.84
2	5.84	5.98	5.89	5.90	5.92	5.83	5.85	5.87
3	6.20	6.29	6.02	6.17	6.19	6.08	6.15	6.14
4	6.11	6.16	5.82	6.03	6.10	5.98	6.12	6.07
5	5.93	5.94	5.73	5.87	6.00	5.97	6.01	5.99
6	5.76	5.81	5.66	5.74	5.92	5.87	5.89	5.89
7	5.83	5.79	5.64	5.75	5.84	5.83	5.85	5.84
8	6.02	6.00	5.88	5.97	6.00	5.93	5.97	5.97
9	5.87	5.90	5.86	5.88	5.86	5.80	5.82	5.83
10	5.90	5.97	6.05	5.97	5.81	5.79	5.76	5.79
11	6.46	6.43	6.59	6.49	5.99	5.97	6.01	5.99
12	6.59	6.54	6.68	6.60	5.84	5.80	5.75	5.80
13	6.87	6.89	7.00	6.92	5.95	5.93	5.86	5.91
14	7.02	6.99	6.99	7.00	5.85	5.81	5.74	5.80
15	7.33	7.39	7.23	7.32	6.08	6.02	5.94	6.01
16	7.27	7.33	7.15	7.25	5.92	5.87	5.78	5.86
17	7.23	7.37	7.22	7.27	5.95	5.87	5.80	5.87
18	7.46	7.53	7.32	7.44	6.07	5.98	5.93	5.99
19	7.45	7.47	7.26	7.39	6.00	5.94	5.91	5.95
20	7.46	7.51	7.29	7.42	6.02	5.93	5.94	5.96
21	7.35	7.45	7.23	7.34	5.98	5.88	5.88	5.91
22	7.41	7.29	7.48	7.39	5.93	5.82	5.84	5.86
23	7.26	7.43	7.19	7.29	5.78	5.74	5.78	5.77
24	7.33	7.45	7.23	7.34	5.89	5.76	5.79	5.81
25	7.51	7.54	7.28	7.44	5.99	5.86	5.86	5.90
26	7.35	7.41	7.19	7.32	5.89	5.75	5.74	5.79
27	7.39	7.54	7.18	7.37	6.00	5.89	5.83	5.91
28	7.22	7.36	7.21	7.26	5.84	5.77	5.80	5.80
29	7.19	7.26	7.11	7.19	5.84	5.65	5.63	5.71
30	7.25	7.37	7.27	7.30	5.93	5.70	5.79	5.81

Table A.20. pH trends in fermenting bottles 1P-3P (40°C, pretreatment, no bromoform) and 1PB-3PB (40°C, pretreatment, bromoform) (batch 2).

	Sample				Sample			
Day	1P	2P	3P	Average (1P-3P)	1PB	2PB	3PB	Average (1PB-3PB)
1	6.65	6.67	6.71	6.68	6.71	6.72	6.73	6.72
2	6.34	6.35	6.40	6.36	6.58	6.34	6.38	6.43
3	6.39	6.49	6.41	6.43	6.81	6.41	6.42	6.55
4	6.32	6.37	6.25	6.31	6.60	6.47	6.52	6.53
5	6.27	6.18	6.15	6.20	6.53	6.35	6.52	6.47
6	6.15	6.11	6.08	6.11	6.26	6.29	6.27	6.27
7	6.03	6.09	6.11	6.08	6.29	6.31	6.36	6.32
8	6.18	6.29	6.27	6.25	6.45	6.43	6.53	6.47
9	6.15	6.23	6.23	6.20	6.44	6.42	6.55	6.47
10	6.27	6.35	6.33	6.32	6.54	6.45	6.65	6.55
11	6.27	6.36	6.32	6.32	6.50	6.44	6.61	6.52
12	6.21	6.41	6.29	6.30	6.41	6.39	6.54	6.45
13	6.29	6.74	6.35	6.46	6.48	6.45	6.59	6.51
14	6.34	7.07	6.43	6.61	6.49	6.49	6.66	6.55
15	6.44	7.27	6.47	6.73	6.55	6.54	6.69	6.59
16	6.44	7.08	6.40	6.64	6.46	6.46	6.61	6.51
17	6.74	7.11	6.39	6.75	6.47	6.45	6.65	6.52
18	7.16	7.14	6.55	6.95	6.58	6.60	6.70	6.63
19	7.22	7.24	6.55	7.00	6.60	6.59	6.71	6.63
20	7.41	7.25	6.62	7.09	6.70	6.69	6.81	6.73
21	7.26	7.24	6.56	7.02	6.62	6.60	6.75	6.66
22	7.27	7.19	6.60	7.02	6.61	6.60	6.76	6.66
23	7.21	7.13	6.73	7.02	6.61	6.61	6.78	6.67
24	7.22	7.14	6.95	7.10	6.68	6.61	6.76	6.68
25	7.37	7.23	7.23	7.28	6.80	6.76	6.91	6.82
26	7.37	7.19	7.37	7.31	6.76	6.74	6.86	6.79
27	7.47	7.28	7.43	7.39	6.95	6.79	6.94	6.89
28	7.47	7.25	7.45	7.39	6.81	6.80	6.90	6.84
29	7.57	7.29	7.53	7.46	6.89	6.83	6.98	6.90
30	7.55	7.20	7.57	7.44	7.02	6.89	7.03	6.98

Table A.21. Dry weight, ash content, and volatile solids (g/mL) in dairy cow manure, pretreated dairy cow manure (2h 100ºC), and fermented dairy cow manure (a=60ºC, b=40ºC) (batch 1).

Dairy Cow Manure

Sample	Dry Weight (g/L)	Ash Content (g/L)	Volatile Solids (g/L)
1	100.20	49.87	50.33
2	100.10	49.75	50.35
3	99.10	49.37	49.73
4	95.00	44.62	50.38
5	95.20	47.50	47.70
6	89.10	36.61	52.49
7	90.90	41.87	49.03
8	99.60	49.79	49.81
9	95.90	46.70	49.20
10	95.20	45.22	49.98
Average	96.03	46.13	49.90
SD (+ or – 1)	3.84	4.28	1.22

Pretreated Dairy Cow Manure (2h 100ºC)

Sample	Dry Weight (g/L)	Ash Content (g/L)	Volatile Solids (g/L)
1	113.50	59.80	53.70
2	119.10	64.74	54.36
3	82.00	44.46	37.54
4	121.60	62.30	59.30
5	133.60	69.00	64.60
6	107.70	57.88	49.82
7	119.90	72.64	47.26
8	119.10	61.80	57.30
9	111.00	56.85	54.15
10	115.80		
Average	114.30	61.05	53.11
SD (+ or – 1)	13.35	8.04	7.73

Fermented Dairy Cow Manure (a=60ºC, b=40ºC)

Sample	Dry Weight (g/L)	Ash Content (g/L)	Volatile Solids (g/L)
1a	233.70	164.08	69.62
2a	232.40	163.61	68.79
3a	193.70	134.10	59.60
4a	248.00	188.18	59.82
5a	209.00	142.25	66.75
6a	198.90	134.00	64.90
Average	219.30	154.37	64.91
SD (+ or – 1)	21.82	21.43	4.35

Table A.21—*Continued*

Fermented Dairy Cow Manure (a=60ºC, b=40ºC)

Sample	Dry Weight (g/L)	Ash Content (g/L)	Volatile Solids (g/L)
1b	189.00	124.91	64.09
2b	180.50	116.68	63.82
3b	177.40	113.84	63.56
4b	218.50	148.65	69.85
5b	194.00	129.96	064.04
6b	175.80	115.87	59.93
Average	189.20	124.98	64.22
SD (+ or – 1)	15.98	13.12	3.19

Table A.22. Dry weight, ash content, and volatile solids (g/mL) in dairy cow manure, pretreated dairy cow manure (2h 100°C), and fermented dairy cow manure (40ºC, 1-3=no pretreatment, no bromoform, 1B-3B=no pretreatment, bromoform, 1P-3P=pretreatment, no bromoform, 1PB-3PB=pretreatment, bromoform) (batch 2).

Dairy Cow Manure

Sample	Dry Weight (g/L)	Ash Content (g/L)	Volatile Solids (g/L)
1	61.30	18.31	42.99
2	57.90	17.65	40.25
3	69.70	31.65	38.05
4	58.10	19.44	38.66
5	77.70	22.42	55.28
6	66.20	28.77	37.43
7	72.80	39.18	33.62
8	66.40	25.49	40.91
9	46.80	15.94	30.86
10	73.70	30.82	42.88
Average	65.06	24.97	40.09
SD (+ or – 1)	9.23	7.54	6.57

Pretreated Dairy Cow Manure (2h 100ºC)

Sample	Dry Weight (g/L)	Ash Content (g/L)	Volatile Solids (g/L)
1	48.60	22.04	26.56
2	58.80	27.90	30.90
3	69.10	35.17	33.93
4	82.00	46.51	35.49
5	47.80	23.18	24.62
6	69.30	36.27	33.03
7	55.70	27.83	27.87
8	55.10	27.37	27.73

Table A.22—*Continued*

Pretreated Dairy Cow Manure (2h 100ºC)			
Sample	Dry Weight (g/L)	Ash Content (g/L)	Volatile Solids (g/L)
9	56.80	29.27	27.53
10	67.90		
Average	61.11	30.62	29.74
SD (+ or – 1)	10.73	7.60	3.73
Fermented Dairy Cow Manure (40ºC, B=bromoform, P=pretreatment)			
Sample	Dry Weight (g/L)	Ash Content (g/L)	Volatile Solids (g/L)
1	107.90	71.15	36.75
2	96.90	63.02	33.88
3	112.60	68.13	44.47
Average	105.80	67.44	38.36
SD (+ or – 1)	8.06	4.11	5.48
1B	112.50	73.16	39.34
2B	129.80	75.06	54.74
3B	108.40	63.72	44.68
Average	116.90	70.65	46.25
SD (+ or – 1)	11.36	6.08	7.8172
1P	112.10	77.38	34.72
2P	104.00	77.18	26.82
3P	115.10	84.02	31.08
Average	110.40	0.07953	30.87
SD (+ or – 1)	5.74	3.89	3.95
1PB	118.40	87.57	30.83
2PB	123.50	90.88	32.62
3PB	109.20	74.16	35.04
Average	117.00	84.20	32.83
SD (+ or – 1)	7.25	8.86	2.11

Table A.23. Dry weight, ash content, and volatile solids (g/mL) in dairy cow manure and pretreated dairy cow manure (2h 100ºC, 7d 40ºC) (batch 3).

Dairy Cow Manure			
Sample	Dry Weight (g/L)	Ash Content (g/L)	Volatile Solids (g/L)
1	98.20	52.54	45.66
2	98.40	54.65	43.75
3	103.80	57.78	46.02
4	99.40	53.69	45.71
5	101.30	54.97	46.33
6	99.60	53.04	46.56
7	100.00	53.99	46.01
8	99.60	52.73	46.87
9	101.60	51.40	50.20
10	104.50	55.55	48.95
Average	100.60	54.03	46.61
SD (+ or – 1)	2.14	1.81	1.80
Pretreated Dairy Cow Manure (2h 100ºC)			
Sample	Dry Weight (g/L)	Ash Content (g/L)	Volatile Solids (g/L)
1a air 10% lime	107.90	60.97	46.93
1b air 10% lime	106.60	62.20	44.40
2a air 10% lime	119.90	68.75	51.15
2b air 10% lime	112.10	63.44	48.66
Average	111.60	63.84	47.78
SD (+ or – 1)	6.00	3.42	2.85
1a no air 10% lime	97.70	54.07	43.63
1b no air 10% lime	117.30	67.13	50.17
Average	107.50	60.60	46.90
SD (+ or – 1)	13.86	9.24	4.62
1a air 5% lime	117.30	64.98	52.32
1b air 5% lime	119.70	65.82	53.88
2a air 5% lime	130.40	74.97	55.43
2b air 5% lime	134.80	77.86	56.94
Average	125.60	70.91	54.64
SD (+ or – 1)	8.39	6.47	1.99
1a no air 5% lime	103.30	55.65	47.65
1b no air 5% lime	110.10	60.44	49.66
Average	106.70	58.05	48.65
SD (+ or – 1)	4.81	3.39	1.42

Table A.23—*Continued*

Pretreated Dairy Cow Manure (7d 40ºC)

Sample	Dry Weight (g/L)	Ash Content (g/L)	Volatile Solids (g/L)
1a air 10% lime	98.00	56.01	41.99
1b air 10% lime	98.40	57.67	40.73
2a air 10% lime	84.20	43.14	41.06
2b air 10% lime	96.10	49.37	46.73
Average	94.18	51.55	42.63
SD (+ or – 1)	6.73	6.66	2.79
1a no air 10% lime	90.90	50.79	40.11
1b no air 10% lime	109.70	62.34	47.36
Average	100.30	56.56	43.74
SD (+ or – 1)	13.29	8.17	5.12
1a air 5% lime	93.50	51.55	41.95
1b air 5% lime	94.40	51.75	42.65
2a air 5% lime	71.70	36.29	35.41
2b air 5% lime	82.00	42.36	39.64
Average	85.40	45.49	39.91
SD (+ or – 1)	10.74	7.53	3.27
1a no air 5% lime	86.00	44.45	41.55
1b no air 5% lime	107.70	57.94	49.76
Average	96.85	51.20	45.65
SD (+ or – 1)	15.34	9.54	5.81

Table A.24. Total and average lime additions (g) to fermenting bottles 1a-6a (60ºC), 1b-6b (40ºC), 1-3 (40ºC, no pretreatment, no bromoform), 1B-3B (40ºC, no pretreatment, bromoform), 1P-3P (40ºC, pretreatment, no bromoform), 1PB-3PB (40ºC, pretreatment, bromoform) from day 1 to 30 (batches 1 and 2).

Sample	Total Lime (g)	Average Lime (g)
1a	52.44	
2a	57.74	
3a	57.98	
4a	60.73	
5a	58.48	
6a	57.89	57.54
1b	41.65	
2b	34.29	
3b	16.13	
4b	26.52	
5b	40.10	
6b	15.38	29.10
1	35.01	
2	35.00	
3	40.01	36.67
1B	30.00	
2B	40.00	
3B	30.00	33.33
1P	35.00	
2P	35.00	
3P	34.99	35.00
1PB	30.00	
2PB	34.99	
3PB	35.00	33.33

Table A.25. Total and average bromoform additions (mL) to fermenting bottles 1a-6a (60ºC), 1b-6b (40ºC), 1-3 (40ºC, no pretreatment, no bromoform), 1B-3B (40ºC, no pretreatment, bromoform), 1P-3P (40ºC, pretreatment, no bromoform), 1PB-3PB (40ºC, pretreatment, bromoform) from day 1 to 30 (batches 1 and 2).

Sample	Total Bromoform Solution (mL)	Average Bromoform Solution (mL)
1a	1.425	
2a	0.475	
3a	0.175	
4a	0.225	
5a	0.150	
6a	0.075	0.420
1b	0.075	
2b	0.075	
3b	0.075	
4b	0.075	
5b	0.175	
6b	0.125	0.100

Sample	Total Bromoform Solution (mL)	Total Pure Bromoform (mL)	Average Bromoform Solution (mL)	Average Pure Bromoform (mL)
1	0	0		
2	0	0		
3	0	0	0	0
1B	4.975	0.350		
2B	4.025	0.350		
3B	2.425	0.250	3.808	0.317
1P	0	0		
2P	0	0		
3P	0	0	0	0
1PB	5.175	0.350		
2PB	3.475	0.350		
3PB	3.575	0.150	4.075	0.283

APPENDIX B

ANOVA Summary Tables

Table B.1. SRP ANOVA summary table (batch 1).

Before Pretreatment and After Pretreatment					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	1955207.93	1	1955208	352.52	2.53×10^{-11} **
Within Groups	77650.28	14	5546.45		
Total	2032858.21	15			
After Pretreatment and After Fermentation (60ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	73872.28	1	73872.28	20.10	7.48×10^{-4} **
Within Groups	44099.23	12	3674.94		
Total	117971.51	13			
After Pretreatment and After Fermentation (40ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	65572.38	1	65572.38	17.86	1.18×10^{-3} **
Within Groups	44052.87	12	3671.07		
Total	109625.24	13			
After Fermentation (60ºC) and After Fermentation (40ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	216.33	1	216.33	0.17	6.90×10^{-1}
Within Groups	12535.99	10	1253.60		
Total	12752.31	11			
Before Pretreatment and After Fermentation (60ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	2453474.95	1	2453475	638.19	8.99×10^{-12} **
Within Groups	46133.40	12	3844.45		
Total	2499608.35	13			
Before Pretreatment and After Fermentation (40ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	2404464.91	1	2404465	626.07	1.01×10^{-11} **
Within Groups	46087.03	12	3840.59		
Total	2450551.94	13			

*Statistically significant at the 5% level
**Statistically significant at the 1% level

Table B.2. Dry Weight ANOVA summary table (batch 1).

Before Pretreatment and After Pretreatment					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	1674.45	1	1674.45	17.35	5.81×10^{-4} **
Within Groups	1736.76	18	96.49		
Total	3411.21	19			
After Pretreatment and After Fermentation (60ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	41307.01	1	41307.01	145.15	8.89×10^{-9} **
Within Groups	3984.11	14	284.58		
Total	45291.12	15			
After Pretreatment and After Fermentation (40ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	21020.69	1	21020.69	102.19	8.17×10^{-8} **
Within Groups	2879.90	14	205.71		
Total	23900.59	15			
After Fermentation (60ºC) and After Fermentation (40ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	2715.02	1	2715.02	7.43	2.14×10^{-2} *
Within Groups	3656.33	10	365.63		
Total	6371.35	11			
Before Pretreatment and After Fermentation (60ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	56967.69	1	5967.69	317.34	5.13×10^{-11} **
Within Groups	2513.19	14	179.51		
Total	59480.88	15			
Before Pretreatment and After Fermentation (40ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	32552.43	1	32552.43	323.45	4.51×10^{-11} **
Within Groups	1408.98	14	100.64		
Total	33961.41	15			

*Statistically significant at the 5% level
**Statistically significant at the 1% level

Table B.3. Ash Content ANOVA summary table (batch 1).

Before Pretreatment and After Pretreatment					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	1054.70	1	1054.70	26.26	8.45×10^{-5} **
Within Groups	682.76	17	40.16		
Total	1737.46	18			
After Pretreatment and After Fermentation (60ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	31348.68	1	31348.68	144.85	2.02×10^{-8} **
Within Groups	2813.55	13	216.43		
Total	34162.23	14			
After Pretreatment and After Fermentation (40ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	14713.53	1	14713.53	138.74	2.61×10^{-8} **
Within Groups	1378.67	13	106.05		
Total	16092.20	14			
After Fermentation (60ºC) and After Fermentation (40ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	2590.63	1	2590.63	8.21	1.68×10^{-2} *
Within Groups	3156.86	10	315.69		
Total	5747.49	11			
Before Pretreatment and After Fermentation (60ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	43933.19	1	43933.19	249.93	2.53×10^{-10} **
Within Groups	2460.95	14	175.78		
Total	46394.14	15			
Before Pretreatment and After Fermentation (40ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	23316.21	1	23316.21	318.13	5.05×10^{-11} **
Within Groups	1026.07	14	73.29		
Total	24342.28	15			

*Statistically significant at the 5% level
**Statistically significant at the 1% level

Table B.4. Volatile Solids ANOVA summary table (batch 1).

Before Pretreatment and After Pretreatment					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	48.96	1	48.96	1.69	2.11×10^{-1}
Within Groups	491.70	17	28.92		
Total	540.66	18			
After Pretreatment and After Fermentation (60ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	501.28	1	501.28	11.37	5.00×10^{-3} **
Within Groups	572.98	13	44.08		
Total	1074.25	14			
After Pretreatment and After Fermentation (40ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	443.79	1	443.79	10.91	5.72×10^{-3} **
Within Groups	529.01	13	40.69		
Total	972.81	14			
After Fermentation (60ºC) and After Fermentation (40ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	1.46	1	1.46	0.10	7.58×10^{-1}
Within Groups	145.54	10	14.55		
Total	147.00	11			
Before Pretreatment and After Fermentation (60ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	845.45	1	845.45	109.34	5.35×10^{-8} **
Within Groups	108.22	14	7.73		
Total	953.67	15			
Before Pretreatment and After Fermentation (40ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	768.76	1	768.76	167.48	3.53×10^{-9} **
Within Groups	64.26	14	4.59		
Total	833.02	15			

** Statistically significant at the 1% level

Table B.5. Total acid and acetic acid ANOVA summary table (batch 1).

After Fermentation (60ºC) and After Fermentation (40ºC)- Total Acid					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	0.0081	1	0.0081	0.0046	9.48×10^{-1}
Within Groups	17.81	10	1.78		
Total	17.82	11			
After Fermentation (60ºC) and After Fermentation (40ºC)- Acetic Acid					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	12.30	1	12.30	13.71	4.09×10^{-3} **
Within Groups	8.97	10	0.90		
Total	21.28	11			

** Statistically significant at the 1% level

Table B.6. CO_2 ANOVA summary table (batch 1).

After Fermentation (60ºC) and After Fermentation (40ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	362270814.70	1	362270814.70	6.52	2.87×10^{-2} *
Within Groups	555692084.40	10	55569208.44		
Total	917962899.10	11			

* Statistically significant at the 5% level

Table B.7. pH ANOVA summary table (batch 1).

After Fermentation (60ºC) and After Fermentation (40ºC)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	0.063	1	0.063	2.12	1.76×10^{-1}
Within Groups	0.30	10	0.030		
Total	0.36	11			

Table B.8. Limestone ANOVA summary table (batch 1).

After Fermentation (60ºC) and After Fermentation (40ºC)

Source of Variation	SS	df	MS	F	P-Value
Between Groups	2441.81	1	2441.81	34.59	1.55×10^{-4} **
Within Groups	705.94	10	70.59		
Total	3147.76	11			

** Statistically significant at the 1% level

Table B.9. Bromoform ANOVA summary table (batch 1).

After Fermentation (60ºC) and After Fermentation (40ºC)

Source of Variation	SS	df	MS	F	P-Value
Between Groups	146302.08	1	146302.08	2.13	1.75×10^{-1}
Within Groups	687604.17	10	68760.42		
Total	833906.25	11			

Table B.10. SRP ANOVA summary table (batch 2).

Before Pretreatment and After Pretreatment					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	3331400.67	1	3331400.67	48.62	6.52×10^{-6} **
Within Groups	959258.71	14	68518.48		
Total	4290659	15			
Before Pretreatment and After Fermentation (1-3)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	1837692.59	1	1837692.59	21.99	1.14×10^{-3} **
Within Groups	752112.06	9	83568.01		
Total	2589804.65	10			
Before Pretreatment and After Fermentation (1B-3B)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	990125.87	1	990125.87	11.77	7.50×10^{-3} **
Within Groups	757120.08	9	84124.45		
Total	1747245.95	10			
After Pretreatment and After Fermentation (1P-3P)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	31029.93	1	31029.93	1.34	2.77×10^{-1}
Within Groups	208876.75	9	23208.53		
Total	239906.68	10			
After Pretreatment and After Fermentation (1PB-3PB)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	9646.49	1	9646.49	0.42	$5.35 \times^{-1}$
Within Groups	208419.84	9	23157.76		
Total	218066.33	10			
After Fermentation (1-3), After Fermentation (1B-3B), After Fermentation (1P-3P), and After Fermentation (1PB-3PB)- 2-Way					
Source of Variation	SS	df	MS	F	P-Value
Pretreatment-No Pretreatment (P-NP)	132018.75	1	132018.75	131.83	< 0.01 **
Bromoform- No Bromoform (B-NB)	66097.36	1	66097.36	66.00	< 0.01 **
P-NP x B-NB Interaction	27458.25	1	27458.25	27.42	< 0.01 **
Within P-NP/B-NB Groups	22574.36	8			

** Statistically significant at the 1% level

Table B.11. Dry weight ANOVA summary table (batch 2).

Before Pretreatment and After Pretreatment

Source of Variation	SS	df	MS	F	P-Value
Between Groups	78.01	1	78.01	0.78	3.89×10^{-1}
Within Groups	1802.59	18	100.14		
Total	1880.61	19			

Before Pretreatment and After Fermentation (1-3)

Source of Variation	SS	df	MS	F	P-Value
Between Groups	3830.19	1	3830.19	47.02	2.74×10^{-5} **
Within Groups	896.08	11	81.46		
Total	4726.27	12			

Before Pretreatment and After Fermentation (1B-3B)

Source of Variation	SS	df	MS	F	P-Value
Between Groups	6201.66	1	6201.66	66.60	5.40×10^{-6} **
Within Groups	1024.24	11	93.11		
Total	7225.90	12			

After Pretreatment and After Fermentation (1P-3P)

Source of Variation	SS	df	MS	F	P-Value
Between Groups	5606.55	1	5606.55	55.95	1.23×10^{-5} **
Within Groups	1102.31	11	100.21		
Total	6708.86	12			

After Pretreatment and After Fermentation (1PB-3PB)

Source of Variation	SS	df	MS	F	P-Value
Between Groups	7217.12	1	7217.12	69.55	4.39×10^{-6} **
Within Groups	1141.42	11	103.77		
Total	8358.54	12			

After Fermentation (1-3), After Fermentation (1B-3B), After Fermentation (1P-3P), and After Fermentation (1PB-3PB)- 2-Way

Source of Variation	SS	df	MS	F	P-Value
Pretreatment-No Pretreatment (P-NP)	16.80	1	16.80	0.24	> 0.05
Bromoform- No Bromoform (B-NB)	235.85	1	235.85	3.38	> 0.05
P-NP x B-NB Interaction	14.96	1	14.96	0.21	> 0.05
Within P-NP/B-NB Groups	267.62	8	69.86		

** Statistically significant at the 1% level

Table B.12. Ash content ANOVA summary table (batch 2).

Before Pretreatment and After Pretreatment					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	151.13	1	151.13	2.64	1.23×10^{-1}
Within Groups	973.74	17	57.28		
Total	1124.86	18			
Before Pretreatment and After Fermentation (1-3)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	4162.06	1	4162.06	83.88	1.77×10^{-6} **
Within Groups	545.80	11	49.62		
Total	4707.86	12			
Before Pretreatment and After Fermentation (1B-3B)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	4815.19	1	4815.19	90.41	1.22×10^{-6} **
Within Groups	585.89	11	53.26		
Total	5401.07	12			
After Pretreatment and After Fermentation (1P-3P)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	5382.90	1	5382.90	109.41	1.05×10^{-6} **
Within Groups	492.01	10	49.20		
Total	5874.91	11			
After Pretreatment and After Fermentation (1PB-3PB)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	6461.14	1	6461.14	104.46	1.30×10^{-6} **
Within Groups	618.55	10	61.85		
Total	7079.69	11			
After Fermentation (1-3), After Fermentation (1B-3B), After Fermentation (1P-3P), and After Fermentation (1PB-3PB)- 2-Way					
Source of Variation	SS	df	MS	F	P-Value
Pretreatment-No Pretreatment (P-NP)	493.41	1	493.41	13.39	< 0.01 *
Bromoform- No Bromoform (B-NB)	46.64	1	46.64	1.27	> 0.05
P-NP x B-NB Interaction	1.61	1	1.61	0.044	> 0.05
Within P-NP/B-NB Groups	541.66	8	36.85		

* Statistically significant at the 5% level
** Statistically significant at the 1% level

Table B.13. Volatile solids ANOVA summary table (batch 2).

Before Pretreatment and After Pretreatment					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	507.70	1	507.70	17.27	6.62×10^{-4} **
Within Groups	499.68	17	29.39		
Total	1007.39	18			
Before Pretreatment and After Fermentation (1-3)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	6.89	1	6.89	0.17	6.89×10^{-1}
Within Groups	448.14	11	40.74		
Total	455.04	12			
Before Pretreatment and After Fermentation (1B-3B)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	87.59	1	87.59	1.88	1.97×10^{-1}
Within Groups	510.38	11	46.40		
Total	597.97	12			
After Pretreatment and After Fermentation (1P-3P)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	2.88	1	2.88	0.20	6.63×10^{-1}
Within Groups	142.75	10	14.27		
Total	145.63	11			
After Pretreatment and After Fermentation (1PB-3PB)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	21.49	1	21.49	1.78	2.11×10^{-1}
Within Groups	120.45	10	12.04		
Total	141.94	11			
After Fermentation (1-3), After Fermentation (1B-3B), After Fermentation (1P-3P), and After Fermentation (1PB-3PB)- 2-Way					
Source of Variation	SS	df	MS	F	P-Value
Pretreatment-No Pretreatment (P-NP)	328.10	1	328.10	11.80	< 0.01 **
Bromoform- No Bromoform (B-NB)	72.73	1	72.73	2.62	> 0.05
P-NP x B-NB Interaction	26.38	1	26.38	0.95	> 0.05
Within P-NP/B-NB Groups	427.21	8	27.79		

** Statistically significant at the 1% level

Table B.14. Total acid and acetic acid ANOVA summary table (batch 2).

After Fermentation (1-3), After Fermentation (1B-3B), After Fermentation (1P-3P), and After Fermentation (1PB-3PB)- Total Acid, 2-Way

Source of Variation	SS	df	MS	F	P-Value
Pretreatment-No Pretreatment (P-NP)	25.87	1	25.87	10.52	< 0.05 *
Bromoform- No Bromoform (B-NB)	37.86	1	37.86	15.39	< 0.01 **
P-NP x B-NB Interaction	4.04	1	4.04	1.64	> 0.05
Within P-NP/B-NB Groups	67.76	8	2.46		

After Fermentation (1-3), After Fermentation (1B-3B), After Fermentation (1P-3P), and After Fermentation (1PB-3PB)- Acetic Acid, 2-Way

Source of Variation	SS	df	MS	F	P-Value
Pretreatment-No Pretreatment (P-NP)	4.66	1	4.66	5.13	> 0.05
Bromoform- No Bromoform (B-NB)	13.45	1	13.45	14.81	< 0.01 **
P-NP x B-NB Interaction	2.77	1	2.77	3.05	> 0.05
Within P-NP/B-NB Groups	20.88	8	0.91		

*Statistically significant at the 5% level
** Statistically significant at the 1% level

Table B.15. CO_2 ANOVA summary table (batch 2).

After Fermentation (1-3), After Fermentation (1B-3B), After Fermentation (1P-3P), and After Fermentation (1PB-3PB)- 2-Way

Source of Variation	SS	df	MS	F	P-Value
Pretreatment-No Pretreatment (P-NP)	11757836.02	1	11757836.02	1.29	> 0.05
Bromoform- No Bromoform (B-NB)	286102315.44	1	286102315.44	31.36	< 0.01 **
P-NP x B-NB Interaction	46720455.87	1	46720455.87	5.12	< 0.05 *
Within P-NP/B-NB Groups	344580607.34	8	9123055.37		

*Statistically significant at the 5% level
** Statistically significant at the 1% level

Table B.16. CH_4 ANOVA summary table (batch 2).

After Fermentation (1-3), After Fermentation (1B-3B), After Fermentation (1P-3P), and After Fermentation (1PB-3PB)- 2-Way					
Source of Variation	SS	df	MS	F	P-Value
Pretreatment-No Pretreatment (P-NP)	49866282.84	1	49866282.84	0.42	> 0.05
Bromoform- No Bromoform (B-NB)	890583157.18	1	890583157.18	7.49	< 0.05 *
P-NP x B-NB Interaction	65880025.56	1	65880025.56	0.55	> 0.05
Within P-NP/B-NB Groups	1006329465.58	8	118966261.52		

*Statistically significant at the 5% level

Table B.17. pH ANOVA summary table (batch 2).

After Fermentation (1-3), After Fermentation (1B-3B), After Fermentation (1P-3P), and After Fermentation (1PB-3PB)- 2-Way					
Source of Variation	SS	df	MS	F	P-Value
Pretreatment-No Pretreatment (P-NP)	1.30	1	1.30	77.66	< 0.01 **
Bromoform- No Bromoform (B-NB)	2.85	1	2.85	170.35	< 0.01 **
P-NP x B-NB Interaction	0.80	1	0.80	47.53	< 0.01
Within P-NP/B-NB Groups	4.95	8			

** Statistically significant at the 1% level

Table B.18. Limestone ANOVA summary table (batch 2).

After Fermentation (1-3), After Fermentation (1B-3B), After Fermentation (1P-3P), and After Fermentation (1PB-3PB)- 2-Way					
Source of Variation	SS	df	MS	F	P-Value
Pretreatment-No Pretreatment (P-NP)	2.11	1	2.11	0.17	> 0.05
Bromoform- No Bromoform (B-NB)	18.84	1	18.84	1.51	> 0.05
P-NP x B-NB Interaction	2.11	1	2.11	0.17	> 0.05
Within P-NP/B-NB Groups	23.06	8	12.49		

Table B.19. Bromoform ANOVA summary table (batch 2).

After Fermentation (1-3), After Fermentation (1B-3B), After Fermentation (1P-3P), and After Fermentation (1PB-3PB)- 2-Way					
Source of Variation	SS	df	MS	F	P-Value
Pretreatment-No Pretreatment (P-NP)	0.00083	1	0.00083	0.20	> 0.05
Bromoform- No Bromoform (B-NB)	0.27	1	0.27	64.80	< 0.01 **
P-NP x B-NB Interaction	0.00083	1	0.00083	0.20	> 0.05
Within P-NP/B-NB Groups	0.27	8	0.0042		

** Statistically significant at the 1% level

Table B.20. SRP ANOVA summary table (batch 3).

Before Pretreatment and After Pretreatment (2h 100ºC, 10% Lime, + Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	3463399.40	1	3463399.40	7.31	2.21×10^{-2} *
Within Groups	4734842.06	10	473484.21		
Total	8198241.47	11			

Before Pretreatment and After Pretreatment (2h 100ºC, 5% Lime, + Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	2251176.99	1	2251176.99	4.79	5.34×10^{-2} *
Within Groups	4698006.03	10	469800.60		
Total	6949183.02	11			

Before Pretreatment and After Pretreatment (2h, 100ºC, 10% Lime, No Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	1013502.24	1	1013502.24	1.73	2.25×10^{-1}
Within Groups	4680938.59	8	585117.32		
Total	5694440.83	9			

Before Pretreatment and After Pretreatment (2h, 100ºC, 5% Lime, No Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	626266.62	1	626266.62	1.08	3.30×10^{-1}
Within Groups	4658418.18	8	582302.27		
Total	5284684.81	9			

Before Pretreatment and After Pretreatment (7d 40ºC, 10% Lime, + Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	1350416.79	1	1350416.79	2.79	1.26×10^{-1}
Within Groups	4837254.19	10	483725.42		
Total	6187670.97	11			

Before Pretreatment and After Pretreatment (7d 40ºC, 5% Lime, + Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	282795.13	1	282795.13	0.57	4.67×10^{-1}
Within Groups	4949888.15	10	494988.82		
Total	5232683.29	11			

Before Pretreatment and After Pretreatment (7d 40ºC, 10% Lime, No Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	158704.32	1	158704.32	0.27	6.19×10^{-1}
Within Groups	4743366.06	8	592920.76		
Total	4902070.38	9			

Table B.20—*Continued*

Before Pretreatment and After Pretreatment (7d 40ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Between Groups	13702.50	1	13702.50	0.024	8.82×10^{-1}
Within Groups	4651011	8	581376.47		
Total	4664714.23	9			

After Pretreatment (2h 100ºC, 10% Lime, + Air), After Pretreatment (2h 100ºC, 5% Lime, + Air), After Pretreatment (2h 100ºC, 10% Lime, No Air), and After Pretreatment (2h 100ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Air-No Air (A-NA)	405660.90	1	405660.90	20.82	< 0.01 **
5%-10% Lime (5-10)	1592955.21	1	152955.21	7.85	< 0.05 *
A-NA x 5-10 Interaction	2559.68	1	2558.68	0.13	> 0.05
Within A-NA/5-10 Groups	561174.78	12	19479.56		

After Pretreatment (7d 40ºC, 10% Lime, + Air), After Pretreatment (7d 40ºC, 5% Lime, + Air), After Pretreatment (7d 40ºC, 10% Lime, No Air), and After Pretreatment (7d 40ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Air-No Air (A-NA)	664013.34	1	664013.34	12.39	< 0.01 **
5%-10% Lime (5-10)	629576.98	1	629576.98	11.75	< 0.01 **
A-NA x 5-10 Interaction	462.90	1	462.90	0.0086	>0.05
Within A-NA/5-10 Groups	1294053.22	12	53589.17		

After Pretreatment (2h 100ºC, 10% Lime, + Air), After Pretreatment (2h 100ºC, 5% Lime, + Air), After Pretreatment (7d 40ºC, 10% Lime, + Air), and After Pretreatment (7d 40ºC, 5% Lime, + Air)

Source of Variation	SS	df	MS	F	P-Value
5%-10% (5-10)	368219.63	1	368219.63	6.48	< 0.05 *
40°C-100°C (40-100)	1042776.31	1	1042776.31	18.36	< 0.01 **
5-10 x 40-100 Interaction	27268.84	1	27268.84	0.48	> 0.05
Within 5-10/40-100 Groups	1438264.78	12	56795.36		

Table B.20—*Continued*

After Pretreatment (2h 100ºC, 10% Lime, No Air), After Pretreatment (2h 100ºC, 5% Lime, No Air), After Pretreatment (7d 40ºC, 10% Lime, No Air), and After Pretreatment (7d 40ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
5%-10% (5-10)	166893.32	1	166893.32	3.42	> 0.05
40°C-100°C (40-100	718943.42	1	718943.42	14.73	> 0.05
5-10 x 40-100 Interaction	28139.32	1	28139.32	0.58	> 0.05
Within 5-10/40-100 Groups	913976.06	4	48822.12		

After Pretreatment (2h 100ºC, 10% Lime, + Air), After Pretreatment (2h 100ºC, 10% Lime, No Air), After Pretreatment (7d 40ºC, 10% Lime, + Air), and After Pretreatment (7d 40ºC, 10% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Air-No Air (A-NA)	548231.85	1	548231.85	14.37	< 0.01 **
40°C-100°C (40-100)	826207.73	1	826207.73	21.65	< 0.01 **
A-NA x 40-100 Interaction	2801.40	1	2801.40	0.073	> 0.05
Within A-NA/40-100 Groups	1377240.98	12	3816.57		

After Pretreatment (2h 100ºC, 5% Lime, + Air), After Pretreatment (2h 100ºC, 5% Lime, No Air), After Pretreatment (7d 40ºC, 5% Lime, + Air), and After Pretreatment (7d 40ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Air-No Air (A-NA)	506030.89	1	506030.89	14.50	< 0.01 **
40°C-100°C (40-100	1719569.80	1	1719569.80	49.26	< 0.01 **
A-NA x 40-100 Interaction	15631.68	1	15631.68	0.45	> 0.05
Within A-NA/40-100 Groups	2241232.37	12	34906.17		

* Statistically significant at the 5% level

** Statistically significant at the 1% level

Table B.21. Dry weight ANOVA summary table (batch 3).

Before Pretreatment and After Pretreatment (2h 100ºC, 10% Lime, + Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	344.77	1	344.77	27.74	1.99×10^{-4} **
Within Groups	149.15	12	12.43		
Total	493.92	13			
Before Pretreatment and After Pretreatment (2h 100ºC, 5% Lime, + Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	1772.88	1	1772.88	84.19	9.00×10^{-7} **
Within Groups	252.69	12	21.06		
Total	2025.57	13			
Before Pretreatment and After Pretreatment (2h 100ºC, 10% Lime, No Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	78.43	1	78.43	3.36	9.67×10^{-2}
Within Groups	233.40	10	23.34		
Total	311.84	11			
Before Pretreatment and After Pretreatment (2h 100ºC, 5% Lime, No Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	61.21	1	61.21	9.50	1.16×10^{-2} *
Within Groups	64.44	10	6.44		
Total	125.65	11			
Before Pretreatment and After Pretreatment (7d 40ºC, 10% Lime, + Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	119.42	1	119.42	8.10	1.48×10^{-2} *
Within Groups	177.01	12	14.75		
Total	296.43	13			
Before Pretreatment and After Pretreatment (7d 40ºC, 5% Lime, + Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	663.69	1	663.59	20.57	6.84×10^{-4} **
Within Groups	387.18	12	32.27		
Total	1050.78	13			
Before Pretreatment and After Pretreatment (7d 40ºC, 10% Lime, No Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	0.19	1	0.19	0.0088	9.27×10^{-1}
Within Groups	218.04	10	21.80		
Total	28.24	11			

Table B.21—*Continued*

Before Pretreatment and After Pretreatment (7d 40ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Between Groups	23.94	1	23.94	0.86	3.74×10^{-1}
Within Groups	276.77	10	27.68		
Total	300.71	11			

After Pretreatment (2h 100ºC, 10% Lime, + Air), After Pretreatment (2h 100ºC, 5% Lime, + Air), After Pretreatment (2h 100ºC, 10% Lime, No Air), and After Pretreatment (2h 100ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Air-No Air (A-NA)	527.85	1	527.85	11.94	< 0.01 **
5%-10% Lime (5-10)	172.27	1	172.27	3.90	> 0.05
A-NA x 5-10 Interaction	216.83	1	216.83	4.91	< 0.05 *
Within A-NA/5-10 Groups	916.94	12	44.20		

After Pretreatment (7d 40ºC, 10% Lime, + Air), After Pretreatment (7d 40ºC, 5% Lime, + Air), After Pretreatment (7d 40ºC, 10% Lime, No Air), and After Pretreatment (7d 40ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Air-No Air (A-NA)	308.88	1	308.88	4.17	> 0.05
5%-10% Lime (5-10)	149.45	1	149.45	2.02	> 0.05
A-NA x 5-10 Interaction	28.36	1	28.36	0.38	> 0.05
Within A-NA/5-10 Groups	486.69	12	74.14		

After Pretreatment (2h 100ºC, 10% Lime, + Air), After Pretreatment (2h 100ºC, 5% Lime, + Air), After Pretreatment (7d 40ºC, 10% Lime, + Air), and After Pretreatment (7d 40ºC, 5% Lime, + Air)

Source of Variation	SS	df	MS	F	P-Value
5%-10% (5-10)	26.52	1	26.52	0.40	> 0.05
40°C-100°C (40-100)	3317.76	1	3317.76	49.72	< 0.01 **
5-10 x 40-100 Interaction	515.29	1	515.29	7.72	< 0.05 *
Within 5-10/40-100 Groups	3859.57	12	66.73		

Table B.21—*Continued*

After Pretreatment (2h 100ºC, 10% Lime, No Air), After Pretreatment (2h 100ºC, 5% Lime, No Air), After Pretreatment (7d 40ºC, 10% Lime, No Air), and After Pretreatment (7d 40ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
5%-10% (5-10)	9.03	1	9.03	0.058	> 0.05
40°C-100°C (40-100)	145.35	1	145.35	0.93	> 0.05
5-10 x 40-100 Interaction	3.51	1	3.51	0.022	> 0.05
Within 5-10/40-100 Groups	157.89	4	156.84		

After Pretreatment (2h 100ºC, 10% Lime, + Air), After Pretreatment (2h 100ºC, 10% Lime, No Air), After Pretreatment (7d 40ºC, 10% Lime, + Air), and After Pretreatment (7d 40ºC, 10% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Air-No Air (A-NA)	4.00	1	4.00	0.079	> 0.05
40°C-100°C (40-100	607.62	1	607.62	11.99	< 0.01 **
A-NA x 40-100 Interaction	105.06	1	105.06	2.07	> 0.05
Within A-NA/40-100 Groups	716.69	12	50.69		

After Pretreatment (2h 100ºC, 5% Lime, + Air), After Pretreatment (2h 100ºC, 5% Lime, No Air), After Pretreatment (7d 40ºC, 5% Lime, + Air), and After Pretreatment (7d 40ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Air-No Air (A-NA)	54.76	1	54.76	0.81	> 0.05
40°C-100°C (40-100	2500.00	1	2500.00	36.96	< 0.01 **
A-NA x 40-100 Interaction	918.09	1	918.09	13.57	< 0.01 **
Within A-NA/40-100 Groups	3472.85	12	67.65		

* Statistically significant at the 5% level

** Statistically significant at the 1% level

Table B.22. Ash content ANOVA summary table (batch 3).

Before Pretreatment and After Pretreatment (2h 100ºC, 10% Lime, + Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	274.89	1	274.89	50.96	1.18×10^{-5} **
Within Groups	64.73	12	5.39		
Total	339.62	13			
Before Pretreatment and After Pretreatment (2h 100ºC, 5% Lime, + Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	813.76	1	813.76	62.87	4.12×10^{-6} **
Within Groups	155.31	12	12.94		
Total	969.07	13			
Before Pretreatment and After Pretreatment (2h 100ºC, 10% Lime, No Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	71.87	1	71.87	6.26	3.14×10^{-2} *
Within Groups	114.88	10	11.49		
Total	186.75	11			
Before Pretreatment and After Pretreatment (2h 100C, 5% Lime, No Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	26.86	1	26.86	6.54	2.85×10^{-2} *
Within Groups	41.06	10	4.11		
Total	67.92	11			
Before Pretreatment and After Pretreatment (7d 40ºC, 10% Lime, + Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	17.67	1	17.67	1.31	2.76×10^{-1}
Within Groups	162.47	12	13.54		
Total	180.14	13			
Before Pretreatment and After Pretreatment (7d 40ºC, 5% Lime, + Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	208.57	1	208.57	12.53	4.07×10^{-3} **
Within Groups	199.77	12	16.65		
Total	408.34	13			
Before Pretreatment and After Pretreatment (7d 40ºC, 10% Lime, No Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	10.69	1	10.69	1.11	3.17×10^{-1}
Within Groups	96.33	10	9.63		
Total	107.02	11			

Table B.22—*Continued*

Before Pretreatment and After Pretreatment (7d 40ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Between Groups	13.39	1	13.39	1.11	3.17×10^{-1}
Within Groups	120.53	10	12.05		
Total	133.92	11			

After Pretreatment (2h 100ºC, 10% Lime, + Air), After Pretreatment (2h 100ºC, 5% Lime, + Air), After Pretreatment (2h 100ºC, 10% Lime, No Air), and After Pretreatment (2h 100ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Air-No Air (A-NA)	259.35	1	259.35	12.26	< 0.01 **
5%-10% Lime (5-10)	20.39	1	20.39	0.96	> 0.05
A-NA x 5-10 Interaction	92.55	1	92.55	4.38	> 0.05
Within A-NA/5-10 Groups	372.29	12	21.15		

After Pretreatment (7d 40ºC, 10% Lime, + Air), After Pretreatment (7d 40ºC, 5% Lime, + Air), After Pretreatment (7d 40ºC, 10% Lime, No Air), and After Pretreatment (7d 40ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Air-No Air (A-NA)	115.11	1	115.11	3.02	> 0.05
5%-10% Lime (5-10)	130.50	1	130.50	3.43	> 0.05
A-NA x 5-10 Interaction	0.48	1	0.48	0.013	> 0.05
Within A-NA/5-10 Groups	246.08	12	38.07		

After Pretreatment (2h 100ºC, 10% Lime, + Air), After Pretreatment (2h 100ºC, 5% Lime, + Air), After Pretreatment (7d 40ºC, 10% Lime, + Air), and After Pretreatment (7d 40ºC, 5% Lime, + Air)

Source of Variation	SS	df	MS	F	P-Value
5%-10% (5-10)	1.02	1	1.02	0.026	> 0.05
40°C-100°C (40-100	1422.50	1	1422.50	36.78	< 0.01 **
5-10 x 40-100 Interaction	172.27	1	172.27	4.45	> 0.05
Within 5-10/40-100 Groups	1595.79	12	38.67		

Table B.22—*Continued*

After Pretreatment (2h 100ºC, 10% Lime, No Air), After Pretreatment (2h 100ºC, 5% Lime, No Air), After Pretreatment (7d 40ºC, 10% Lime, No Air), and After Pretreatment (7d 40ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
5%-10% (5-10)	31.35	1	31.35	0.49	> 0.05
40°C-100°C (40-100)	59.22	1	59.22	0.93	> 0.05
5-10 x 40-100 Interaction	3.96	1	3.96	0.062	> 0.05
Within 5-10/40-100 Groups	94.53	4	63.65		

After Pretreatment (2h 100ºC, 10% Lime, + Air), After Pretreatment (2h 100ºC, 10% Lime, No Air), After Pretreatment (7d 40ºC, 10% Lime, + Air), and After Pretreatment (7d 40ºC, 10% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Air-No Air (A-NA)	3.16	1	3.16	0.12	> 0.05
40°C-100°C (40-100)	266.68	1	266.68	10.12	< 0.01 **
A-NA x 40-100 Interaction	68.24	1	68.24	2.59	> 0.05
Within A-NA/40-100 Groups	338.08	12	26.35		

After Pretreatment (2h 100ºC, 5% Lime, + Air), After Pretreatment (2h 100ºC, 5% Lime, No Air), After Pretreatment (7d 40ºC, 5% Lime, + Air), and After Pretreatment (7d 40ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Air-No Air (A-NA)	51.16	1	51.16	1.56	> 0.05
40°C-100°C (40-100	1041.28	1	1041.28	31.68	< 0.01 **
A-NA x 40-100 Interaction	344.93	1	344.93	10.49	< 0.01 **
Within A-NA/40-100 Groups	1437.37	12	32.87		

*Statistically significant at the 5% level
** Statistically significant at the 1% level

Table B.23. Volatile solids ANOVA summary table (batch 3).

Before Pretreatment and After Pretreatment (2h 100ºC, 10% Lime, + Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	3.95	1	3.95	0.89	3.64×10^{-1}
Within Groups	53.39	12	4.45		
Total	57.34	13			
Before Pretreatment and After Pretreatment (2h 100ºC, 5% Lime, + Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	184.39	1	184.39	53.98	8.89×10^{-6} **
Within Groups	40.99	12	3.42		
Total	225.39	13			
Before Pretreatment and After Pretreatment (2h 100ºC, 10% Lime, No Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	0.14	1	0.14	0.028	8.70×10^{-1}
Within Groups	50.46	10	5.05		
Total	50.60	11			
Before Pretreatment and After Pretreatment (2h 100ºC, 5% Lime, No Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	6.98	1	6.98	2.24	1.65×10^{-1}
Within Groups	31.10	10	3.11		
Total	38.07	11			
Before Pretreatment and After Pretreatment (7d 40ºC, 10% Lime, + Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	45.22	1	45.22	10.35	7.39×10^{-3} **
Within Groups	52.41	12	4.37		
Total	97.62	13			
Before Pretreatment and After Pretreatment (7d 40ºC, 5% Lime, + Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	128.10	1	128.10	25.14	3.02×10^{-4} **
Within Groups	61.14	12	5.10		
Total	189.24	13			
Before Pretreatment and After Pretreatment (7d 40ºC, 10% Lime, No Air)					
Source of Variation	SS	df	MS	F	P-Value
Between Groups	13.75	1	13.75	2.48	1.46×10^{-1}
Within Groups	55.33	10	5.53		
Total	69.07	11			

Table B.23—*Continued*

Before Pretreatment and After Pretreatment (7d 40ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Between Groups	1.52	1	1.52	0.24	6.33×10^{-1}
Within Groups	62.80	10	6.28		
Total	64.32	11			

After Pretreatment (2h 100ºC, 10% Lime, + Air), After Pretreatment (2h 100ºC, 5% Lime, + Air), After Pretreatment (2h 100ºC, 10% Lime, No Air), and After Pretreatment (2h 100ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Air-No Air (A-NA)	47.21	1	47.21	10.20	< 0.01 **
5%-10% Lime (5-10)	74.13	1	74.13	16.01	< 0.01 **
A-NA x 5-10 Interaction	26.06	1	26.06	5.63	< 0.05 *
Within A-NA/5-10 Groups	147.39	12	4.63		

After Pretreatment (7d 40ºC, 10% Lime, + Air), After Pretreatment (7d 40ºC, 5% Lime, + Air), After Pretreatment (7d 40ºC, 10% Lime, No Air), and After Pretreatment (7d 40ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Air-No Air (A-NA)	46.87	1	46.87	5.05	< 0.05 *
5%-10% Lime (5-10)	0.64	1	0.64	0.069	> 0.05
A-NA x 5-10 Interaction	21.47	1	21.47	2.32	> 0.05
Within A-NA/5-10 Groups	68.99	12	9.28		

After Pretreatment (2h 100ºC, 10% Lime, + Air), After Pretreatment (2h 100ºC, 5% Lime, + Air), After Pretreatment (7d 40ºC, 10% Lime, + Air), and After Pretreatment (7d 40ºC, 5% Lime, + Air)

Source of Variation	SS	df	MS	F	P-Value
5%-10% (5-10)	17.14	1	17.14	2.25	> 0.05
40°C-100°C (40-100)	395.37	1	395.37	51.82	< 0.01 **
5-10 x 40-100 Interaction	91.68	1	91.68	12.02	< 0.01 **
Within 5-10/40-100 Groups	504.19	12	7.63		

After Pretreatment (2h 100ºC, 10% Lime, No Air), After Pretreatment (2h 100ºC, 5% Lime, No Air), After Pretreatment (7d 40ºC, 10% Lime, No Air), and After Pretreatment (7d 40ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
5%-10% (5-10)	6.73	1	6.73	0.32	> 0.05
40°C-100°C (40-100)	19.02	1	19.02	0.91	> 0.05
5-10 x 40-100 Interaction	0.013	1	0.013	0.00064	> 0.05
Within 5-10/40-100 Groups	25.76	4	20.83		

Table B.23—*Continued*

After Pretreatment (2h 100ºC, 10% Lime, + Air), After Pretreatment (2h 100ºC, 10% Lime, No Air), After Pretreatment (7d 40ºC, 10% Lime, + Air), and After Pretreatment (7d 40ºC, 10% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Air-No Air (A-NA)	0.050	1	0.050	0.0065	> 0.05
40°C-100°C (40-100)	69.22	1	69.22	9.11	< 0.05 *
A-NA x 40-100 Interaction	3.96	1	3.96	0.52	> 0.05
Within A-NA/40-100 Groups	73.22	12	7.60		

After Pretreatment (2h 100ºC, 5% Lime, + Air), After Pretreatment (2h 100ºC, 5% Lime, No Air), After Pretreatment (7d 40ºC, 5% Lime, + Air), and After Pretreatment (7d 40ºC, 5% Lime, No Air)

Source of Variation	SS	df	MS	F	P-Value
Air-No Air (A-NA)	0.061	1	0.061	0.00097	> 0.05
40°C-100°C (40-100)	314.39	1	314.39	49.86	< 0.01 **
A-NA x 40-100 Interaction	137.54	1	137.54	21.81	< 0.01 **
Within A-NA/40-100 Groups	451.99	12	6.31		

* Statistically significant at the 5% level

** Statistically significant at the 1% level

WORKS CITED

Adams, Todd, Nancy Easterling, and Anne McFarland. 2005. *Semiannual water quality report for the North Bosque River watershed and Lake Waco* (TR0508). Stephenville, Texas Institute for Applied Environmental Research, Tarleton State University. Database on-line. Available from http://www.tiaer.tarleton.edu.

Aiello-Mazzarri, Cateryna, Frank K. Agbogbo, and Mark T. Holtzapple. 2006. Conversion of municipal solid waste to carboxylic acids using a mixed culture of mesophilic microorganisms. *Bioresource Technology*, 97:47-56.

American Public Health Association. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th ed. Washington, DC: American Public Health Association.

Barnett, G.M. 1994. Phosphorus forms in animal manure. *Bioresource Technology*, 49:139-147.

Brintrup, R., T. Mooren, U. Meyer, H. Spiekers, and E. Pfeffer. 1993. Effects of two levels of phosphorus intake on performance and faecal phosphorus excretion of dairy cows. *Journal of Animal Physiology and Animal Nutrition*, 69:29-36.

Callaghan, F.J., D.A.J. Wase, K. Thayanithy, and C.F. Forster. 1999. Co-digestion of waste organic solids: batch studies. *Bioresource Technology*, 67:117-122.

Carpenter, S. R., N.R. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley, and V.H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications*, 8(3):559-568.

Castleberry, V. 2001. *Determination of volatile organic compound (VOC) content of Aspen wood in biomass experiments, repeat II (ashing)*. Experimental File #107. Waco, Baylor University, Environmental Studies Lab 127.

Chang, Vincent S., Barry Burr, and Mark T. Holtzapple. 1997. Lime pretreatment of switchgrass. *Applied Biochemistry and Biotechnology*, 63-65:3-19.

Chang, Vincent.S., William E. Kaar, Barry Burr, and Mark T. Holtzapple. 2001a. Simultaneous saccharification and fermentation of lime-treated biomass. *Biotechnology Letters*, 23:1327-1333.

Chang, Vincent S., Murlidhar Nagwani, Chul-Ho Kim, and Mark T. Holtzapple. 2001b. Oxidative lime pretreatment of high-lignin biomass. *Applied Biochemistry and Biotechnology*, 94:1-25. .

City of Waco, Texas. 2003. *Water quality issues*. Database on-line. Available from http://www.waco-texas.com/city_depts/waterutilities/waterissues.htm.

Davies, J.M, M. Roxborough, and A. Mazumder. 2004. Origins and implications of drinking water odours in lakes and reservoirs of British Columbia, Canada. *Water Research*, 38(7):1900-1910.

Dou, Z., J.D. Ferguson, J. Fiorini, J.D. Toth, S.M. Alexander, L.E. Chase, C.M. Ryan, K.F. Knowlton, R.A. Kohn, A.B. Peterson, J.T. Smith, and Z.Wu. 2003. Phosphorus feeding levels and critical control points on dairy farms. *Journal of Dairy Science*, 86(11):3787-3795.

Dou, Zhengxia, Katharine F. Knowlton, Richard A. Kohn, Zhiguo Wu, Larry D. Satter, Gangya Zhang, John D. Toth, and James D. Ferguson. 2002. Phosphorus characteristics of dairy feces affected by diets. *Journal of Environmental Quality*, 312058-2065.

Dou, Z., Toth, J.D., Galligan, D.T., Ramberg, C.F., Jr., & Ferguson, J.D. 2000. Laboratory procedures for characterizing manure phosphorus. *Journal of Environmental Quality*, 29:508-514.

Doyle, Robert D. (n.d.). SRP (PO_4) determination, low concentration PO_4 procedures. Doyle's Laboratory Procedures. Waco, Baylor University.

Doyle, R.D., J.T. Scott, and T. Conry (n.d.). *Planktonic nitrogen fixation in a Texas reservoir: hot spots and hot moments*. Oral. Waco, Baylor University.

Ebeling, Angela M., Larry G. Bundy, J. Mark Powell, and Todd W. Andraski. 2002. Dairy diet phosphorus effects on phosphorus losses in runoff from land-applied manure. *Soil Science Society of America Journal*, 66:284-291.

Gandi, Jagruti, Mark T. Holtzapple, Alexis Ferrer, F. Michael Byers, Nancy D. Turner, Murlidhar Nagwani, and Sushien Chang. 1997. Lime treatment of agricultural residues to improve rumen digestibility. *Animal Feed Science Technology*, 68:195-211.

Gerardi, Michael H. 2003. *The Microbiology of Anaerobic Digesters*. Hoboken: Wiley-Interscience.

Ghaly, A.E. 1996. A comparative study of anaerobic digestion of acid cheese whey and dairy manure in a two-stage reactor. *Bioresource Technology*, 58:61-72.

Hart, J., M. Gangwer, and E.S. Marx. 1997. Dairy manure as a fertilizer source. *Nutrient Management for Dairy Production* (EM 8586). Oregon: Oregon State University, Extension Service.

Hauck, Larry. 2002 *Investigations of phosphorus enrichment and control in the Lake Waco-Bosque River watershed: an overview of technical and stakeholder aspects* (PR0204). Stephenville, Texas Institute for Applied Environmental Research, Tarleton State University. Database on-line. Available from http://www.tiaer.tarleton.edu.

Hinrichs, Roger A., and Merlin Kleinbach. 2002. *Energy, its use and the environment*. Edited by J. Vondeling, P. McGahey, R. Bonner, and D.L. Passek. Fort Worth: Harcourt College Publishers.

Holtzapple, Mark.T., Richard R. Davison, M. Kyle Ross, Salvador Aldrett-Lee, Murlidhar, Nagwani, Chang-Ming Lee, Champion Lee, Seth Adelson, William Kaar, David Gaskin, Hiroshi Shirage, Nan-Sheng Chang, Vincent S. Change, and Mitchell E. Loesher. 1999. Biomass conversion to mixed alcohol fuels using the MixAlco process. *Applied Biochemistry and Biotechnology*, 77-79:609-631.

Kaar, William E., and Mark T. Holtzapple. 2000. Using lime pretreatment to facilitate the enzymic hydrolysis of corn stover. *Biomass and Bioenergy*, 18:189-199.

Kalff, Jacob. 2002. *Limnology*. Edited by T. Ryu & J. Hakin. Upper Saddle River: Prentice Hall.

Kim, Sehoon, and Mark T. Holtzapple. 2006. Effect of structural features on enzyme digestibility of corn stover. *Bioresource Technology*, 97:583-591.

Kim, Youngsug, Yeongho Lee, Chai C. Gee, and Euiso Choi. 1997. Treatment of taste and odor causing substances in drinking water. *Water Science Technology*, 35(8):29-36.

Koschel, R.H. 1997. Structure and function of pelagic calcite precipitation in lake ecosystems. *Verhandlungen der Internationalen Vereinigung fürTheoretische und Angewandte Limnologie*, 26:343-349. In Prepas et al. 2001.

Koutsoukos, Petros G. n.d. *Current knowledge of calcium phosphate chemistry and in particular solid surface-water interference interactions*. Patras, Greece: University of Patras, Institute of Chemical Engineering and High Temperature Chemical Processes and Department of Engineering.

Leatham, David J., John F. Schmucker, Ronald D. Lacewell, Robert B. Schwart, Ashley C. Lovell, and Greg Allen. 1992. Our industry today, Impact of Texas water quality laws on dairy income and viability. *Journal of Dairy Science*, 75:2846-2856.

Lind, Christopher. 2002. Livestock waste management and lake rehabilitation. *Clearwaters*, 32(1):1-8. Database on-line. Available from http://www.nywea.org/clearwaters/321030.html.

Martin, Angela, and G. Dennis Cooke. 1994. Health risks in eutrophic water supplies. *Lakeline*, 14 (April), 24-26.

McFarland, A., Kiesling, R., Hauck, L., & Matlock, M. (n.d.). *Linking chemical and biological monitoring components in the TMDL process*. Database on-line. Available from http://www.nqmc.org/2000proceeding/papers/pap_mcfarland.pdf.

McFarland, Anne, Richard Kiesling, and Chris Pearson. 2001. *Characterization of a Central Texas reservoir with emphasis on factors influencing algal growth* (TR0104). Stephenville, Texas Institute for Applied Environmental Research, Tarleton State University. Database on-line. Available from http://www.tiaer.tarleton.edu.

McFarland, Anne, Ali Saleh, and Larry Hauck. 2000. *Demonstration of phosphorus best management practices in the North Bosque River basin* (TR0002). Stephenville, Texas Institute for Applied Environmental Research, Tarleton State University. Database on-line. Available from http://www.tiaer.tarleton.edu.

McFarland, Anne M.S., and Larry M. Hauck. 1999. Relating agricultural land uses to in-stream stormwater quality. *Journal of Environmental Quality*, 28(3):836-844. Journal on-line. Available from http://firstsearch.odc.org/.

McFarland, Anne .M.S., and Larry M. Hauck. 2001. Determining nutrient export coefficients and source loading uncertainty using in-stream monitoring data. *Journal of the American Water Resources Association*, 37(1):223-236.

Mickey, Ruth M., Olive Jean Dunn, and Virginia A. Clark. 2004. *Applied Statistics; Analysis of Variance and Regression*. 3rd ed. Edited by D.J Balding, N.A.C.Cressie, N.I. Fisher, I.M. Johnstone, J.B. Kadane, G. Molenberghs, L.M. Ryan, D.W. Scott, A.F.M. Smith, and J.L. Teugels. Hoboken: John Wiley & Sons, Incorporated.

Miller, G.Tyler, Jr. 2000. *Living in the environment*. 11th ed. Edited by K. Milotich & S. Lussier. Pacific Grove: Brooks/Cole Publishing Company.

Morse, D., H.H. Head, C.J. Wilcox, H.H. Van Horn, C.D. Hissem, and B. Harris, Jr. 1992. Effects of concentration of dietary phosphorus on amount and route of excretion. *Journal of Dairy Science*, 75:3039-3049.

Murphy, Sheila. 2002. *General information on phosphorus*. City of Boulder, United States Geological Survey Water Quality Monitoring. Database on-line. Available from http://bcn.boulder.co.us/basin/data/NUTRIENTS/info/TP.html.

National Research Council. 1989. *Nutrient requirements of dairy cattle*. 6th ed., rev. Washington D.C.: National Academy Press. In Satter and Wu 1999.

Odom, E. Dale. 2004. *Dairy Cattle*. Red River Authority of Texas. Database on-line. Available from http://www.rra.dst.tx.us/bi/mammal/DAIRY%20CATTLE.dfm.

Olmstead, J., III and Williams, G.M. 1994. *Chemistry: the molecular science*. Edited by J.M. Smith, J.S. Murdzek, and P. Joiner. St. Lois: Mosby-Year Book, Incorporated.

Pierzynski, G.M. 2000. *Methods for P Analysis*. Raleigh: North Carolina State University, Department of Soil Science. Database on-line. Available from http://www.soil.ncsu.edu/seral17/publications/ser17-2/p_methods2000.pdf.

Prepas, Ellie E., Bernadette Pinel-Alloul, Patricia A. Chambers, Tom P. Murphy, Sharon Reedyk, Greg Sandland, and Mark Serediak. 2001. Lime treatment and its effects on the chemistry and biota of hardwater eutrophic lakes. *Freshwater Biology*, 46:1049-1060.

Ristinen, Robert A., and Jack J. Kraushaar. 1999. *Energy and the environment*. Edited by S. Johnson and S. Russell. New York: John Wiley & Sons, Incorporated.

Ross, M.Kyle, and Mark T. Holtzapple. 2001. Laboratory method for high-solids countercurrent fermentations. *Applied Biochemistry and Biotechnology*, 94:111-126.

Satter, L.D., & Wu, Z. 1999. Phosphorus nutrition of dairy cattle-what's new? In *Proceedings of the Cornell Nutrition Conference for Feed Manufacturers*. Ithaca, Departments of Animal Science and Poultry Science, New York State College of Agriculture and Life Sciences.

Scott, Thad, and Anne McFarland. 2002. *Water quality trends in the Lake Waco-Bosque River watershed, an interim update* (TR0207). Stephenville, Texas Institute for Applied Environmental Research, Tarleton State University. Database on-line. Available from http://www.tiaer.tarleton.edu.

Sharpley, Andrew N., S.C. Chapra, R. Wedepohl, J.T. Sims, T.C. Daniel, and K.R. Reddy. 1994. Managing agricultural phosphorus for protection of surface waters: issues and options. *Journal of Environmental Quality*, 23:437-451.

Smith, Val H., Jonathan Sieber-Denlinger, Frank deNoyelles, Jr., Scott Campbell, Shugen Pan, Stephen J. Randtke, Gerald T. Blain, and Vernon A. Strasser. 2002. Managing taste and odor problems in a eutrophic drinking water reservoir. *Lake and Reservoir Management*, 18(4):319-323.

Spiekers, H., R. Brintrup, M. Balmelli, and E. Pfeffer. 1993. Influence of dry matter intake on faecal phosphorus losses in dairy cows fed rations low in phosphorus. *Journal of Animal Physiology and Animal Nutrition*, 69:37-43. In Dou et al. 2002 and Wu, Satter, and Sojo 2000.

Sullivan, S., D. Thomas, W. Wlliott, and S. Segura. 1995. *Volumetric survey of Waco Lake*. Austin, Texas Water Development Board, Hydrographic Survey Group. In McFarland, Kiesling, and Pearson 2001.

Svendsen, Elin Kyhl and T. Henry Blackburn. 1986. Sequential phases in the anaerobic degradation of swine manure. *Agricultural Wastes*, 16:47-65.

Texas Commission on Environmental Quality. 1992. *State of Texas Clean Water Act Section 303(d) List*. Database on-line. Available from http://www.tceq.tx.us/assets/public/compliance/monops/water/92_303d.pdf.

Texas Commission on Environmental Quality. 1994. *State of Texas Clean Water Act Section 303(d) List*. Database on-line. Available from http://www.tceq.tx.us/assets/public/compliance/monops/water/94_303d.pdf.

Texas Commission on Environmental Quality. 1996. *State of Texas Clean Water Act Section 303(d) List*. Database on-line. Available from http://www.tceq.tx.us/assets/public/compliance/monops/water/96_303d.pdf.

Texas Commission on Environmental Quality. 1998. *State of Texas Clean Water Act Section 303(d) List*. Database on-line. Available from http://www.tceq.tx.us/assets/public/comm_exec/pubs/sfr/058_98/98_303d.pdf.

Texas Commission on Environmental Quality. 1999. *State of Texas Clean Water Act Section 303(d) List*. Database on-line. Available from http://www.tceq.tx.us/assets/public/comm_exec/pubs/sfr/058_99/99_303d.pdf.

Texas Commission on Environmental Quality. 2000. *State of Texas Clean Water Act Section 303(d) List*. Database on-line. Available from http://www.tceq.tx.us/assets/public/compliance/monops/water/00_303d.pdf.

Texas Commission on Environmental Quality. 2002. *State of Texas Clean Water Act Section 303(d) List*. Database on-line. Available from http://www.tceq.tx.us/assets/public/compliance/monops/water/02twqmar/02_303d.pdf.

Texas Commission on Environmental Quality. 2004. *State of Texas Clean Water Act Section 303(d) List* [draft]. Database on line. Available from http://www.tceq.tx.us/assets/public/compliance/monops/water/04twqi/04_303d.pdf.

Texas Commission on Environmental Quality and Texas State Soils and Water Conservation Board. 2003. *Reducing phosphorus in the North Bosque River, Taking action to improve water quality* (GI-306). Database on-line. Available from http://www.tceq.state.tx.us/comm_exec/forms_pubs/pubs/gi/gi-306_1072021.pdf.

Texas Natural Resource Conservation Commission. 2001. *Two total maximum daily loads for phosphorus in the North Bosque River, for segments 1226 and 1255*. Austin, The Strategic Assessment Division. Database on-line. Available from http://www.tnrcc.state.tx.us/water/quality/tmdl/bosque_tmdl.pdf.

Texas State Historical Association. 2001. *The North Bosque River*. The handbook of Texas online. Austin, University of Texas. Database on-line. Available from http://www.tsha.utexas.edu/handbook/online/articles/NW/rnns.html.

Texas Natural Resource Conservation Commission. 2001. *Two total maximum daily load for phosphorus in the North Bosque River*. Austin, The Strategic Assessment Division.

Thanakoses, Piyarat, Nagat Abd Alla Mostafa, and Mark T. Holtzapple. 2003. Conversion of sugarcane bagasse to carboxylic acids using a mixed culture of mesophilic microorganisms. *Applied Biochemistry and Biotechnology*, 105-108:523-546.

Turner, J. Rick, and Julian F. Thayer. 2001. *Introduction to analysis of variance*. Edited by C.D. Laughton, E. Carr, S. Robinson, and C. Bear. Thousand Oaks: Sage Publications.

United States Department of Agriculture. 2004a. *Texas State and county data, census of agriculture*. National Agriculture Statistics Service (Volume 1, Geographic Area Series, AC-02-A-43A). Database on-line. Available from http://www.nass.usda.gov/census/.

United States Department of Agriculture. 2004b. *United States summary and state data, census of agriculture*. National Agriculture Statistics Service (Volume 1, Geographic Area Series, AC-02-A-51). Database on-line. Available from http://www.nass.usda.gov/census/.

Van Loon, Gary.W., & Stephen J. Duffy. 2000a. *Environmental Chemistry*. New York: Oxford University Press.

Van Walusm, G.P. 1999. *Solid Dry Weight*. Standard Operating Procedures #3. Waco, Baylor University, Environmental Studies Lab 127.

Virginia Technical Institute. 1996. *Dairy manure management: how much of individual nutrients are excreted by dairy cows?* Blacksburg, Virginia Technical Institute, Department of Dairy Science. Database on-line. Available from http://www.dasc.vt.edu/nutritioncc/9638b.html.

Walker, William.W., Jr. 1983. Significance of eutrophication in water supply reservoirs. *Journal of the American Water Works Association*, 75:38-42.

Weast, R.C., and M.J. Astle, eds. 1978. *Handbook of Chemistry and Physics*. 59th ed. Boca Raton: CRC Press, Incorporated.

Wu, Z., L.D. Satter, and R. Sojo. 2000. Milk production, reproductive performance, and fecal excretion of phosphorus by dairy cows fed three amounts of phosphorus. *Journal of Dairy Science*, 83:1028-1041.