PRETREATMENT AND FERMENTATION OF SUGARCANE TRASH TO CARBOXYLIC ACIDS

A Thesis

by

BALASUBRAMANIYAN NACHIAPPAN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2008

Major Subject: Chemical Engineering

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Approved by:

Chair of Committee,	Mark Holtzapple
Committee Members,	Charles Glover
	Raghupathy Karthikeyan
Head of Department,	Michael Pishko

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ABSTRACT

Pretreatment and Fermentation of Sugarcane Trash to Carboxylic Acids.

(December 2008)

Balasubramaniyan Nachiappan, B.Sc., Texas Tech University

Chair of Advisory Committee: Dr. Mark Holtzapple

The rising price of oil is hurting consumers all over the world. There is growing interest in producing biofuels from non-food crops, such as sugarcane trash. Lignocellulosic biomass (e.g., sugarcane trash) is an abundant, inexpensive, and renewable resource. The patented MixAlco process is a cost-effective solution, which does not require sterility or the addition of expensive enzymes to convert lignocellulosic biomass to transportation fuels and valuable chemicals. In this study, the MixAlco process was used to convert sugarcane trash to carboxylic acids under thermophilic conditions.

Lime-treated sugarcane trash (80%) and chicken manure (20%) was used as the feedstock in rotary 1-L fermentors. Ammonium bicarbonate buffer was used to mitigate the effects of product (carboxylic acid) inhibition. Marine inoculum was used because of the high adaptability of the mixed culture of microorganisms present. Iodoform solution was added to inhibit methanogenesis.

Preliminary batch studies over a 20-day period produced 19.7 g/L of carboxylic acids. Sugarcane trash had the highest average yield (0.31 g total acid/g VS fed) and

highest average conversion (0.70 g VS digested/g VS fed) among the three substrates compared.

Countercurrent fermentations were performed at various volatile solid loading rates (VSLR) and liquid residence times (LRT). The highest acid productivity of 1.40 g/(L·d) was at a total acid concentration of 29.9 g/L. The highest conversion and yield were 0.64 g VS digested/g VS fed and 0.36 g total acid/g VS fed, respectively. The continuum particle distribution model (CPDM) was used to predict acid concentration at various VSLR and LRT. The average error in between the predicted and experimental acid concentration and conversion were 4.62% and 1.42%, respectively.

The effectiveness of several pretreatment methods was evaluated using the CPDM method. The best-performing method was short-term, no-wash, oxidative lime pretreatment with ball milling. At an industrial-scale solids loading of 300 g VS/L liquid, the CPDM "map" predicts a total acid concentration of 64.0 g/L at LRT of 30 days, VSLR of 7 g/(L·d), and conversion of 57%. Also high conversion of 76% and high acid concentration of 52 g/L are achieved at a VSLR of 4 g/(L·d) and LRT of 30 days.

DEDICATION

I would like to dedicate this thesis to God and my loving parents. I really want to thank my father, for his financial support and his sincere words of wisdom and my mother, for her continuous moral support and strong encouragement. Without God's grace and my parents support, I would not have been able to complete this work successfully.

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I would like to thank Dr. Cesar Granda for always being there as my mentor. He was always willing to clarify my doubts and provide advice. I would like to thank Dr. Zihong Fu, who taught me the principles of CPDM before graduation. Another important person I would like to thank is Lakshmi Muthiah, who has been very supportive and helpful throughout graduate school. I would also like to thank Andrea Forrest and Rocio Sierra Ramirez for teaching me lab procedures and helping me get started with my research. I would also like to thank all the other lab group members, Aaron Smith, Hema Rughoonundun, JiPeng Dong, Matthew Falls, Sebastian Taco Vasquez, Michael Landoll, and Somsak Watanawanavet for their wonderful friendship.

I would like to thank the engineering technician, Randy Marek for helping to fix and construct laboratory equipment used in this research work. Finally, I would like to thank all the secretaries and staff members of Artie Mcferrin Department of Chemical Engineering for the great service they have provided throughout my studies.

NOMENCLATURE

Aceq	Acetic acid equivalent concentration (g acetic acid equivalents/L)
a	Parameter constant (g acetic acid equivalent/L)
b	Parameter constant (g acetic acid equivalent/(L·d))
c	Parameter constant (d^{-1})
e	Parameter constant (g acetic acid equivalent/(g VS·d))
f	Parameter constant (dimensionless)
g	Parameter constant (L/g total acid)$^{1/h}$
h	Parameter constant (dimensionless)
LRT	Liquid residence time (days)
VSLR	Volatile solids loading rate (g VS/(L·d))
S_0	Initial substrate concentration (g VS/L)
s	Selectivity (g total acid produced/g VS digested)
σ	Selectivity (g aceq produced/g VS digested)
ϕ	(g total acids/g acetic acid equivalents)
x	Conversion (g VS digested/g VS fed)
r	Reaction rate (g acetic acid equivalents/(L·d))
$\hat{r}$	Specific reaction rate (g acetic acid equivalents produced/(g VS·d))
$\hat{r}_{pred}$	Predicted spec. reaction rate (g acetic acid equivalents produced/(g VS·d))
α	Acetic acid equivalent concentration (mol acetic acid equivalents/L)
LTW	Long-term wash (long-term air-lime pile pretreatment)

LTNW	Long-term no-wash (long-term, submerged, air-lime pretreatment)
STW	Short-term wash (short-term, acid-wash, oxidative lime pretreatment)
STNW	Short-term no-wash (short-term, no-wash, oxidative lime pretreatment)
STW-BM	Short-term wash ball-milled (short-term, acid-wash, oxidative lime pretreatment with ball milling)
STNW-BM	Short-term no-wash ball-milled (short-term, no-wash, oxidative lime pretreatment with ball milling)

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CHAPTER I

INTRODUCTION

This chapter discusses the global energy crisis. Biofuels can greatly diversify the nation's fuel supply. As background, current biomass conversion technologies and various types of biofuels are discussed. This chapter introduces lignocellulosic biomass as a promising feedstock for future biofuel production and discusses the challenges facing lignocellulosic ethanol. The research focus is on the MixAlco process, a low-cost versatile technology that converts lignocellulosic biomass (e.g., sugarcane trash) into biofuels. The objectives of this research work are highlighted. As additional background, various biomass pretreatment methods are described. Lastly, some background is given on countercurrent anaerobic fermentation and the Continuum Particle Distribution Model (CPDM), which is used to predict product concentrations and conversions.

1.1 Need for Sustainable Energy Resources

We are living in a period of uncertainty with rising energy prices. The era of cheap oil has ended. The price of crude oil is currently about $120 per barrel and it seems like $4.00 per gallon gasoline is here to stay. Throughout the 1990's, oil was stable around $30 per barrel (Kirby and Cambell, 2008). Since then, there has been a four-fold increase in oil prices. There are many possible reasons for this increase. There are a lot of conflicts in oil-rich regions, such as Nigeria and the Middle East.

This thesis follows the style and format of Bioresource Technology.

There are increasing concerns over the supplies and reserves available as well. In addition, fossil fuel reserves are dwindling. Furthermore, there has been a mammoth increase in oil demand, mainly from developing countries, such as China and India. According to the International Energy Agency (2007), global energy demand is expected to increase 50% by 2030, and 45% of that increase will be in China and India alone. Most of the "easy" oil reserves have already been discovered and tapped. The remaining reserves are relatively inaccessible and there are high costs associated with extracting crude oil from these areas.

The most controversial and significant environmental concern with burning fossil fuels is the release of carbon dioxide. Carbon dioxide is an important greenhouse gas. The Intergovernmental Panel on Climate Change (IPCC) has reported that most of the observed increase in global temperatures is very likely due to the observed increases in anthropogenic greenhouse gas concentrations (IPCC, 2007). This has led to the ratification of the Kyoto Protocol in 1997. The goal of this international treaty is to lower worldwide emissions of carbon dioxide and five other greenhouse gases (Oberthur and Ott, 1999).

Another potential problem is the accumulation of waste products (e.g., agricultural waste, animal manure, and municipal solid waste). Some of which can cause health problems. Unless efficient disposal techniques are utilized, accumulation of waste could pose serious problems in areas with high population densities.

1.2 Sustainable Liquid Transportation Fuel

The use of biomass energy can reduce dependence on foreign oil because biofuels are a form of renewable liquid transportation fuels. Biofuels have the potential to replace about 30% of current gasoline consumption on a sustainable basis (Perlack et al., 2005). Biomass (plant-derived matter) represents 47% of total renewable energy consumption and is the single largest renewable energy source. Plants capture sunlight and convert carbon dioxide to carbohydrates via photosynthesis. Biofuels are carbon neutral because the carbon dioxide released during combustion is fixed again during photosynthesis. Biofuels can help diversify the nation's transportation fuels. Biofuels are not new; they have been used for more than a century. The internal combustion engine and the diesel engine were initially designed to run on alcohol fuels. Ford's famous Model T was designed to run on ethanol, gasoline, or both. Henry Ford even described ethanol as the fuel of the future and built an ethanol production plant in the Midwest (Pahl, 2005). Because of low-cost petroleum, biofuel production ended in the United States. After the OPEC oil crisis in the 1970's, there was renewed interest in biofuels and there was increased research in that area. Currently, the United States is the world's leading producer of ethanol. In 2007, the United States increased its production by 33% to 24.5 billion liters of ethanol, accounting for about half of the world production of 54 billion liters (Monfort, 2008).

1.3 Types of Biofuels and Biomass Conversion Technology

There are three generations of biofuels. First-generation biofuels are derived from sugar (e.g., sugarcane), starch (e.g., corn), vegetable oils (e.g., soybeans), and animal fats (e.g., chicken). Corn is currently the most widely used biomass source of biofuels in the United States. In Brazil, sugarcane-derived ethanol is the most widely used. Grain-based biomass is usually converted to ethanol using the sugar platform. Usually, enzymes convert starch to sugars. The sugars are then fermented using microorganisms, such as yeast, to produce ethanol. The ethanol has to be distilled and dehydrated. In most cities, ethanol blends up to 10% are readily available. E85 (85% ethanol, 15% gasoline) requires engine modification and currently works only for flex-fuel vehicles. Ethanol is also used to produce an oxygenated fuel additive, ethyl butyl ether (ETBE), which is formed in the reaction between ethanol and isobutylene. Ethanol transportation is not possible with existing pipelines because ethanol tends to absorb water and impurities found in pipelines, which adversely affects engine performance. The sugar platform suffers from the need for sterility, limited availability, competition with food, and difficult separations. Biodiesel is produced from transesterification of vegetable oils and fats. The end-products are alkyl esters (biodiesel) and glycerol. Biodiesel is typically blended with commercial diesel fuel in concentrations between 5 to 20%.

Second-generation biofuels use a variety of non-food crops. Biomass sources include crop residues, forest residues, dedicated energy crops, municipal solid waste, and other forms of cellulosic biomass. Converting cellulosic biomass has generally been

uneconomical because of the high cost of enzymes and the extensive pretreatment required. Increased research in this area has significantly reduced the cost of production and is projected to be a significant part of United States biofuel production by 2020. Biofuels can also be produced using a thermochemical platform. This involves gasifying the biomass to carbon monoxide and hydrogen (syngas). Syngas is then converted to synthetic fuels using gas-to-liquid conversion technology, such as the Fischer-Tropsch process. The thermochemical platform also has disadvantages such as 30–40% biomass energy lost to heat, low conversions, expensive gasifiers, and complex downstream processing.

Third-generation biofuel is derived from algae. Algae can produce 30 times more energy per acre than land crops and they have much faster growth rates than terrestrial crops. Using micro-algae to produce biofuels might be the only viable way to replace gasoline in the United States (Sheehan and Benneman, 1998).

The following figures were adapted from the U.S. Department of Energy's website. Figure 1-1 shows the reduction in greenhouse gas emissions for various types of fuels and Figure 1-2 shows the energy input for producing one Btu of fossil fuel energy. It is clear that cellulosic biomass has a very clear advantage in both these areas, accounting for 86% reduction in greenhouse gases and about 90% net energy output. Figure 1-3 shows the projected U.S. biofuel sources. Although currently most of the ethanol production comes from corn, Figure 1-3 shows that for sustainable production, non-food sources such as crop residues, forest waste, and perennial energy crops would account for most of the biofuel production in the future.

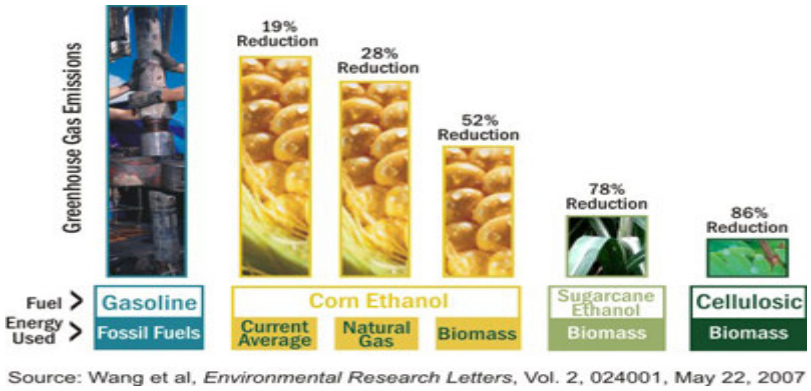


Figure 1-1. Greenhouse gas reduction (Source: Wang et al., 2007).

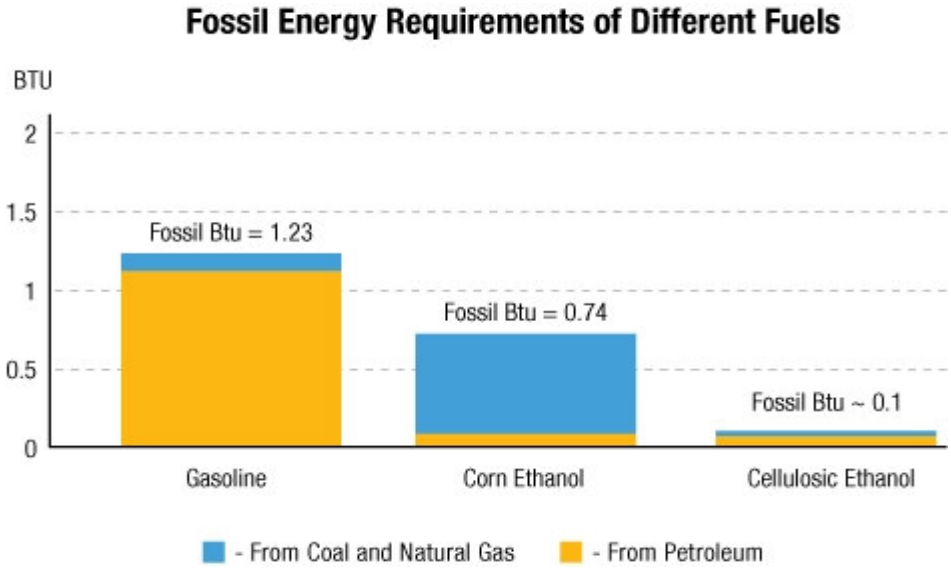


Figure 1-2. Fossil energy requirements of different fuels (Source: Wang et al., 2007).

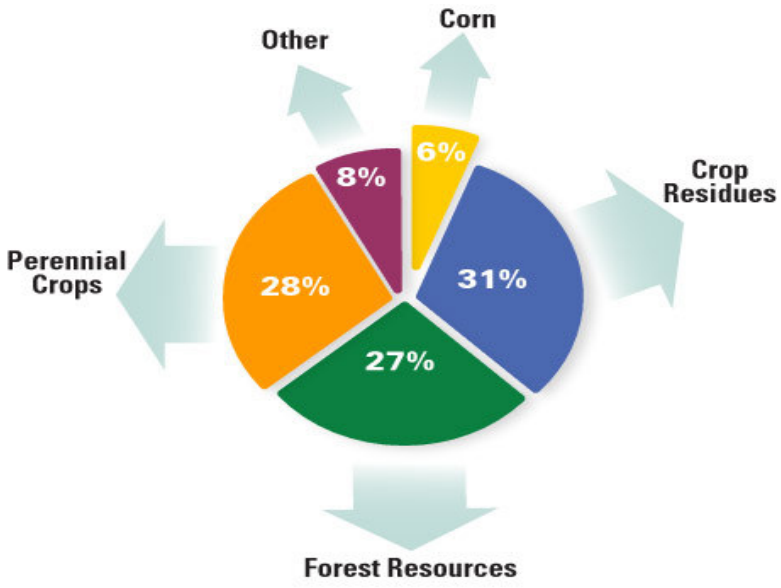


Figure 1-3. Projected U.S. biofuel sources (Source: Perlack et al., 2005).

1.4 Lignocellulosic Biomass

The United States, which produces ethanol primarily from corn, and Brazil, which primarily uses sugarcane, account for 95% of the world's ethanol production. However, both these feedstocks are currently expensive and compete with food supplies. Corn future prices in the United States are expected to be in the range of $4.95–$5.35 per bushel up to March 2010 (Hart, 2008). An alternative feedstock, which eliminates the problems presented above, is lignocellulosic biomass. Currently the government is focusing on funding research to develop cost-effective technologies to produce cellulosic ethanol. Lignocellulosic biomass accounts for 50% of the biomass in the world (Claassen et al., 1999) and is inexpensive. Ethanol from lignocellulosic biomass has the

potential to contribute substantially to bioethanol for transportation (Ragauskas et al., 2006). Lignocellulosic biomass is composed of forest residues, municipal solid waste, agricultural residues, and dedicated energy crops (Lin and Tanaka, 2005). Fermentation of lignocellulosic biomass is environmentally friendly and is also an attractive way to dispose of agricultural and industrial wastes. Some of the dedicated energy crops, such as switchgrass, energy cane, and miscanthus, provide high biomass yields and can be harvested several times a year.

1.5 Composition of Lignocellulosic Biomass

Lignocellulose consists of cellulose, hemicellulose, and lignin. Cellulose is the main constituent of plant cell walls. Cellulose consists of D-glucopyranose monomer units bound by β-1-4-glycosidic linkages. The average degree of polymerization (DP) for cellulose ranges from 500 to 15000 (Holtzapple, 1993). Hydrogen bonds and van der Waals's forces between cellulose molecules result in parallel alignment and crystalline structure (Zhang and Lynd, 2004). In addition to the crystalline region, there is also a less ordered region called the amorphous region. The amorphous region allows easier disintegration of cellulose by hydrolysis compared to the crystalline region. Hemicellulose polymers are shorter than cellulose polymers with a lower degree of polymerization (DP) 50–200. It provides the linkage between cellulose and lignin. Hemicellulose is a polysaccharide composed of three hexoses (glucose, galactose, and mannose) and two pentoses (xylose and arabinose). Lignin is a phenylpropane-based polymer and is the largest non-carbohydrate fraction of lignocellulose. It consists of

coniferyl alcohol, sinapyl alcohol, and coumaryl alcohol monomers. An important characteristic of lignin is that it cannot be depolymerized to its original monomers. Lignin gives structural rigidity and its hydrophobic nature prevents water loss from the vascular tissues of plants. Lignin and hemicellulose form a sheath that protects the cellulosic portion of biomass (Holtzapple, 1993).

1.6 Challenges for Lignocellulosic Ethanol

The crystalline structure of cellulose – as well as the complex structural organization of cellulose, hemicellulose, and lignin – makes lignocellulosic material difficult to decompose. Pretreatment is necessary to alter the structure of lignocellulosic biomass and make it more digestible. Sugar monomers can be produced from cellulose and hemicellulose either by acids or by hydrolytic enzymes. Presently, enzymatic hydrolysis is considered the most promising technology for converting biomass into sugars. However, the cost of these enzymes is high (Gnansounou and Dauriat, 2005). The glucose produced from cellulose hydrolysis can be easily metabolized by conventional yeast, *Saccharomyces cerevisiae.* However, hemicellulose consists of both hexoses and pentoses. Pentoses are five-carbon sugars and they cannot be efficiently handled by existing microorganisms. Genetically modified organisms that handle five-carbon sugars have been developed, but the cost and ethanol yield do not make pentose fermentation economically attractive.

In conclusion, some of the weaknesses of the sugar platform include strict fermentation conditions such as sterility, limited availability, competition with food, and

difficult separations. Genetically modified organisms are required to efficiently handle the five-carbon sugars. The thermochemical platform requires expensive gasifiers and 30–40% of the energy is lost to heat. A process that eliminates the problems mentioned above and offers a low-cost technology for converting biomass to useful chemicals and fuels is desired. One such process is the MixAlco process, a robust process that does not require sterility and can use all biodegradable components (e.g., carbohydrates, proteins, fats, and pectins). The MixAlco process utilizes the carboxylate platform.

1.7 MixAlco Process (Carboxylate Platform)

The MixAlco process (Holtzapple et al., 1999) is a proven low-cost technology for converting non-food feedstocks into chemicals and fuels. This technology has received numerous patents. There is currently a pilot plant operating in College Station, TX with capacity of about 100 dry pounds per day using feedstock such as paper waste and chicken manure. Currently, construction is ongoing for a semi-works demonstration plant in Bryan, TX to test the scaled-up commercial feasibility of the MixAlco process. The semi-works demonstration plant will have a loading capacity of 400 dry tons of biomass. The main feedstock will be sorghum. The MixAlco process uses the carboxylate platform (Equation 1-2) in contrast to the sugar platform (Equation 1-1) and thermochemical platform. Based on Gibbs energy change, acetic acid production (Equation 1-2) is more favorable for microorganisms compared to ethanol production (Equation 1-1).

Sugar Platform

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2 \qquad \Delta G = -48.56 \text{ kcal/mol} \qquad (1\text{-}1)$$

Carboxylate Platform

$$C_6H_{12}O_6 \rightarrow 3\ CH_3COOH \qquad \Delta G = -61.8 \text{ kcal/mol} \qquad (1\text{-}2)$$

The first step in the MixAlco process (Figure 1-4) involves lime pretreatment to make the biomass more digestible (Chang and Holtzapple, 2000; Fan et al., 1982). The MixAlco process employs anaerobic fermentation of lignocellulosic biomass using a mixed culture of microorganisms. The carboxylic acids produced are neutralized with a buffer and dewatered before being thermally converted to ketones. The ketones can be hydrogenated to produce mixed secondary alcohols (e.g., isopropanol). Alternatively, esterification before hydrogenation can be used to produce mixed primary alcohols (e.g., ethanol) as well. It is also possible to convert the carboxylate salts into carboxylic acids (e.g., acetic acid). This demonstrates the versatility of the process and the wide variety of products possible.

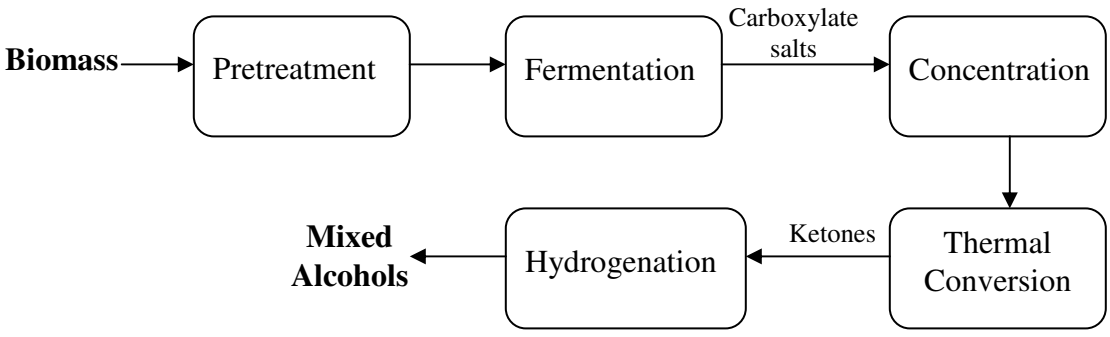


Figure 1-4. Different unit operations in the MixAlco process to produce mixed alcohols from biomass waste.

The MixAlco process can use a wide variety of feedstocks, such as municipal solid waste, sewage sludge, forest product residues, agricultural waste, and non-food energy crops. The mixed culture of microorganisms can be either from terrestrial sources or marine sources. Because they are found under natural conditions, they can adapt easily and do not require sterile conditions to survive. The MixAlco process also does not require the addition of expensive enzymes or genetically modified organisms.

1.8 Sugarcane Trash (Tops and Leaves)

The sugarcane plant is a tall perennial grass. It can grow from 8 to 20 feet tall and it is a native to warm and tropical regions of the world. It can be harvested 4–5 times before replanting. The sugarcane plant consists of 75–80% cane (stalks) from which juice is extracted. The other 20–25% consists of leafy material including tops (Legendre, 2000). The leafy material and tops is collectively known as *trash*. The primary use for sugarcane is sugar production, which is converted to various other products. Sugarcane is rich in sucrose. Sugarcane production in Brazil during the 2007 to 2008 period is expected to be 547.2 million tons (Navarro, 2007). Half of the sugarcane produced in Brazil is used to produce ethanol. The United States produces about 24.7 million tons of sugarcane (Shapouri, 2006). Currently, most of the ethanol produced in the United States is from corn. The major states that produce sugarcane are Louisiana, Texas, Hawaii, and Florida. In 2009, Louisiana will have the first three converted sugar mills producing ethanol from sugarcane (Reyes, 2008). Sugarcane trash is conventionally burned before harvest, or left to rot. This is done so as to avoid the extra costs associated with

transportation and processing. The trash itself does not contribute much in term of sugar, so it is not economically attractive to incur the extra cost. However, sugarcane trash is a lignocellulosic biomass and can be a valuable feedstock for conversion to liquid fuels. The purpose of this research was to determine the productivity of sugarcane trash in the MixAlco process. We believe that sugarcane trash combined with bagasse could be an efficient way to produce lignocellulosic ethanol. Bagasse is the fibrous plant material that remains after crushing and extracting juice from the cane stalks. Extensive research has already been done to evaluate bagasse in the MixAlco process (Thanakoses, 2002; Agbogbo, 2005; Fu, 2007). Table 1-1 shows the compositional differences between sugarcane trash and bagasse used in this research.

Table 1-1. Compositional properties for sugarcane trash and bagasse

Compositional Analysis on dry Basis		
Component	Sugarcane trash	Bagasse*
Glucan	36.2%	42.6%
Xylan	24.0%	23.1%
Arabinan	2.50%	1.52%
Lignin	24.6%	24.1%
Ash	9.70%	4.04%
Others (proteins, extractives, etc)	3%	4.66%

* Bagasse composition from Department of Energy Database for feedstock composition.

1.9 Research Objectives

Sugarcane trash, an underutilized resource, is a potential feedstock in the MixAlco process. Traditionally it is burned before harvest. The smoke associated with burning can have adverse effects on the environment and cause health concerns. The MixAlco process is a low-cost technology for converting lignocellulosic biomass, such

as sugarcane trash, to valuable chemicals and fuels. This research accomplished the following:

1) Performed preliminary batch experiments to evaluate sugarcane trash potential.
2) Performed continuous countercurrent fermentations using sugarcane trash and chicken manure under thermophilic conditions. Countercurrent fermentations mitigate the effects of end-product inhibition and also help to characterize the performance of the substrate in an industrial setting.
3) Validated Continuum Particle Distribution Model (CPDM) results by comparing with experimental results from countercurrent experiments.
4) Evaluated fermentation performance of different combinations of short-term oxidative lime pretreatment and long-term air-lime pretreatment using CPDM.

1.10 Biomass Pretreatment

The digestibility of lignocellulosic biomass is low due to structural features such as lignin, acetyl groups, and crystallinity (Chang and Holtzapple, 2000). Hence, pretreatment is necessary to convert lignocellulosic biomass to liquid fuels. The purpose of pretreatment is to alter or remove the physical and chemical impediments that inhibit the accessibility of enzymes to the substrate (Inoue et al., 2008). The goal of pretreating lignocellulosic biomass is shown in Figure 1-5. Lignin and crystallinity have major impacts on biomass digestibility, whereas acetyl groups have low impact (Chang and Holtzapple, 2000). Pretreatment has been regarded as one of the most expensive steps in

converting biomass to liquid fuel with costs as high as $0.30 per gallon of ethanol produced (Mosier et al., 2005).

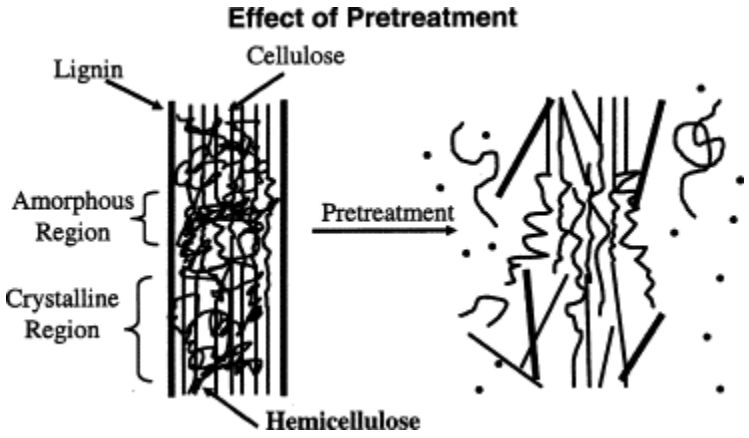


Figure 1-5. Schematic of goal of pretreatment for lignocellulosic biomass (adapted from Hsu et al., 1980).

Ball milling has been reported to increase the digestibility of various lignocellulosic substrates by reducing the crystallinity (Millett et al., 1979). However, ball milling requires high energy input and is not economically feasible. Chemical pretreatments using alkali agents are effective for herbaceous crops and agricultural residues. Alkaline pretreatment breaks the bond between lignin and carbohydrates and disrupts the lignin structure (Kim and Holtzapple, 2006). Alkali pretreatments can be performed at lower temperatures and pressures compared to other pretreatments, such as dilute acid and steam explosion (Mosier et al., 2005).

Lime pretreatment has proven to be a useful chemical method for selectively de-lignifying lignocellulosic biomass, thereby increasing its biodigestibility. As a

pretreatment agent, lime is advantageous because it is inexpensive, $0.06/kg (Miller, 2001); safe to handle; and can be simply recovered (Chang et al., 1998). In lime pretreatment, the biomass is pretreated with calcium hydroxide and water under different conditions of temperature and pressure. Some traditional forms of lime pretreatment are long-term air-lime pretreatment which takes approximately 1 to 2 months. Long-term air-lime pretreatment allows for large amounts of biomass to be pretreated at once. It has about 70% recovery and it degrades some of the sugars mainly from the hemicellulose fraction. This is performed at mild temperatures ranging from 55°C to 65°C and air is supplied at atmospheric pressure. Kim (2004) showed that long-term pretreatment removes about half of the lignin and all the acetyl groups in corn stover. There are two forms of short-term pretreatments. Hot lime-water pretreatment involves boiling a mixture of biomass, lime, and distilled water for 1–3 hours in a large pan (Chang et al., 1997, 1998). The mixture is then allowed to cool down and neutralized using carbon dioxide. This pretreatment was not investigated in this study. This pretreatment removes about a third of the lignin and all the acetyl groups from the biomass (Chang and Holtzapple, 2000). Short-term oxidative pretreatment was another type of pretreatment investigated. The addition of air/oxygen greatly improves the delignification of biomass, especially for high lignin (>25%) biomass (Chang and Holtzapple, 2000). Sugarcane trash has a lignin content of 25%, so it is beneficial to use this form of pretreatment. Short-term pretreatments are usually performed under temperatures ranging from 100°C to 180°C and oxygen pressures ranging from 0.791 to 2.86 MPa (100–400 psig). This pretreatment can vary anywhere from a few minutes to up to 10 hours. Short-term

oxidative pretreatments (Chang et al., 2001) save time and limit sugar degradation. Short-term oxidative lime pretreatment of poplar wood (25–30% lignin) removed 78% of the lignin originally present (Chang et al., 2001).

1.11 Countercurrent Fermentation and CPDM

High product concentration and conversion can be obtained with countercurrent fermentation (Ross and Holtzapple, 2001). In the laboratory, countercurrent experiments were performed in 1-L centrifuge bottles. Figure 1-6 shows the pilot-scale countercurrent fermentors. Fresh biomass is added to the fermentor with the highest carboxylate salt concentration and fresh liquid medium is added to the fermentor with the most digested biomass (Figure 1-7). Liquid is transferred upstream from F4 to F1 and solids are transferred downstream from F1 to F4. Countercurrent fermentation addresses the issues of inhibition due to high concentration of carboxylate salts, as well as the recalcitrant nature of biomass as the reactive portions are digested (Ross and Holtzapple, 2001). Methanogenesis is inhibited by the presence of ammonium ions or the addition of an inhibitor, such as iodoform.

Figure 1-6. Photograph of pilot scale countercurrent fermentors in College Station, TX.

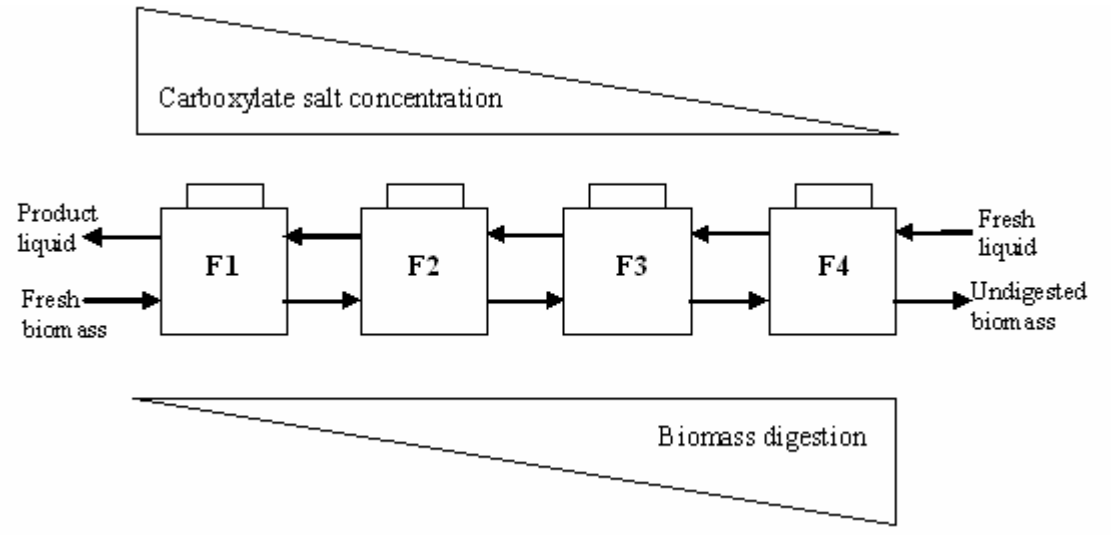


Figure 1-7. Four-stage countercurrent fermentation (F1–F4).

Countercurrent fermentations in the laboratory require a long time to reach steady state (approximately 4 months); therefore, it would be very time consuming and uneconomical to investigate a wide variety of operation conditions to determine the optimum conditions. Loescher (1996) developed the Continuum Particle Distribution Model (CPDM) to overcome this. CPDM allows the prediction of acid concentrations and conversions based on data collected from batch experiments. CPDM has been found to predict acid concentrations and conversions with less than 20% error (Fu, 2007; Agbogbo, 2005; Aiello-Mazzarri, 2002; Thanokoses, 2002). CPDM can save time in determining the optimal operating conditions.

CHAPTER II

MATERIALS AND METHODS

2.1 Substrates

Lime-treated sugarcane trash, lime-treated bagasse, and paper were used as the carbon source for anaerobic fermentations. Chicken manure was used as the nutrient source for the fermentations. Typically an 80% biomass and 20% chicken manure ratio was used.

Raw sugarcane trash was provided by the LSU Audubon Sugar Institute. This was hammer milled at Cater Mattil Hall, Texas A&M University. The sugarcane trash was then pretreated using either long-term air-lime pretreatment or short-term oxidative pretreatment.

Long-term air-lime pretreated bagasse from a previous PhD student's research (Fu, 2007) was used in the initial batch experiments. The raw bagasse was originally obtained from the Lower Rio Grande Valley and ground with a Thomas Wiley Laboratory Mill, Texas A&M University. The bagasse was treated with air/lime pretreatment for six weeks at 50°C. The average moisture content was 0.054 g water/g of wet bagasse and the average ash content was 0.12 g ash/g of dry bagasse. The average volatile solids (VS) content of the lime-treated bagasse was 0.880 g VS/g of dry bagasse (0.760 g of carbohydrates/g of dry bagasse and 0.120 g of lignin/g of dry bagasse). The amount of ash in raw sugarcane trash (9.70%) is more than twice the amount of ash present in raw bagasse (4.04%), as shown in Table 1-1. This could be because the sugarcane trash was not fresh when received. Also, bagasse is washed with water during

sugar extraction, so water-soluble ash is removed.

Chicken manure was obtained from the Poultry Science Center (Texas A&M University, College Station, TX). The chicken manure was oven dried at 105°C for two days and stored in Ziploc bags for future use. The ash and volatile solids composition varied among the different batches of chicken manure collected and dried (Table 2-1).

Waste copier paper was used as the control for the batch experiements. The paper was shredded into small pieces of equal size. No additional treatment methods were used because paper pulping already treats the paper for lignin content. The average moisture content of paper was 0.05 g of water/g of wet paper and the average ash content was 0.133 g of ash/g of dry paper. The average VS content was 0.867g VS/g of dry paper.

Compositional properties such as moisture, VS, ash, and lignin content vary depending on the biomass and the pretreatment as well as neutralization method. Table 2-1 presents all the above properties for all the substrates used in this research. Table 2-1 also has material labels to make identification of certain substrates easier in the following chapters.

For all the substrates, volatile solids were determined by the methodology presented in Ross (1998). The ash content was determined by ashing in a muffle furnace overnight at 550°C (NREL, 2005). The moisture content was determined by drying in an oven at 105°C (NREL, 2008a). The lignin and sugar content was determined as given by NREL Standard Procedure – Determination of Structural Carbohydrates and Lignin in Biomass (NREL, 2008a).

Table 2-1. Compositional properties of the various substrates

Biomass	Pretreatment method	Material label	Moisture content (g water/g wet biomass)	Volatile solids content (g VS/g dry biomass)	Ash content (g ash/g dry biomass)	*Overall lignin content (g lignin/g dry biomass)
Sugarcane trash	Untreated	N/A	0.09	0.903	0.097	0.246
Waste paper	Pretreatment not required	N/A	0.05	0.867	0.133	Not Determined
Sugarcane trash	Long-term, wash, air-lime pretreatment (pile)	**LTW** (Long-term wash)	0.07	0.66 (batch 1) 0.61 (batch 2)	0.34 (batch 1) 0.39 (batch 2)	0.124
Sugarcane trash	Long-term, no-wash, air-lime pretreatment (submerged)	**LTNW** (Long-term no-wash)	0.08	0.605	0.395	0.135
Sugarcane trash	Short-term, acid-wash, oxidative lime pretreatment	**STW** (Short-term wash)	0.103	0.90	0.10	0.139
Sugarcane trash	Short-term, no-wash, oxidative lime pretreatment	**STNW** (Short-term no-wash)	0.11	0.60	0.40	0.139
Sugarcane trash	Short-term, acid-wash, oxidative lime pretreatment (Ball-Milled)	**STW-BM** (Short-term wash ball-milled)	0.04	0.90	0.10	0.139
Sugarcane trash	Short-term, no-wash, oxidative lime pretreatment (Ball-Milled)	**STNW-BM** (Short-term no-wash ball-milled)	0.04	0.60	0.40	0.139

*Note to determine overall lignin content, all samples were acid neutralized and washed as procedure does not allow more than 10% ash.

Table 2-1. (Continued)

Biomass	Pretreatment method	Material label	Moisture content (g water/g wet biomass)	Volatile solids content (g VS/g dry biomass)	Ash content (g ash/g dry biomass)	*Overall lignin content (g lignin/g dry biomass)
Sugarcane bagasse	Long-term, no-wash, air-lime pretreatment (Pile)	N/A	0.054	0.88	0.12	0.120
Chicken manure	None (Used for batch and countercurrent Experiments)	N/A	0.04	0.50	0.50	N/A
Chicken manure	None (2nd batch used for CPDM batch experiments only)	N/A	0.03	0.56	0.44	N/A

*Note to determine overall lignin content, all samples were acid neutralized and washed as procedure does not allow more than 10% ash.

2.2 Biomass Pretreatment

Chemical and physical pretreatments were investigated in this research. Long-term air-lime and short-term oxidative chemical pretreatments were studied. Two different methods of performing long-term air-lime pretreatment procedures were utilized. The methods were long-term pile pretreatment (Appendix A) and long-term submerged pretreatment (Appendix B). Both pretreatments are described in the following sections. For the long-term pile pretreatment, the pretreated biomass was neutralized with carbon dioxide and washed with distilled water to remove the soluble products. For the long-term submerged pretreatment, the pretreated biomass together with the pretreatment liquor was neutralized with carbon dioxide and air dried with no washing. This was done to investigate the effect of washing on the fermentation. Washing could remove some of the degraded volatile solids and decrease the total acid concentration when fermented. Conditions and operating procedures for the short-term oxidative pretreatments are also described in the following sections.

Ball milling was the tested physical pretreatment. It reduces the crystallinity of the biomass substrate making it more digestible (Millett et al., 1979).

2.2.1 Long-term Pile Pretreatment

Long-term air-lime pile pretreatment (Figure 2-1) at 50°C using excess lime (0.4 g $Ca(OH)_2$/g dry biomass) was performed in a storage bin (L × W × H = 0.91 m × 0.61 m × 0.61 m) filled with big PVC pipes in the bottom of a 12.7 cm rock bed. The tub was separated in two sections by a mesh screen, which prevented the rocks from falling over. The other section was filled with distilled water to about ¾ height of the tub. Biomass (4

kg on a dry basis) was mixed well with 1600 g of $Ca(OH)_2$ and a pile was formed on top of the rock bed (Figure 2-2). The water was continuously distributed through the biomass by water sprayers above the pile (Figure 2-3), and was recycled through a water heater. Fresh distilled water was added when required to maintain the liquid level constant. A heat exchanger maintained the biomass treatment system at a constant temperature of 50˚C. Air at 32 normal cubic meter per hour was scrubbed through a lime slurry flask (Figure 2-4) and then bubbled through the pile via air diffusers beneath the pile. The system was monitored daily for leaks and the strainer in the sump discharge line was checked weekly to ensure that it was not clogged. Solid samples were removed weekly from the system to track the lignin degradation. At the end of five weeks, the pretreatment liquor was analyzed for sugar content to account for dissolved sugars. The wet pretreated biomass was recovered from the storage bin and allowed to cool to room temperature. The biomass was stored in several 18.9-L buckets and neutralized using carbon dioxide. A mechanical stirrer was used to ensure the biomass was well mixed. The neutralized biomass was then washed a couple of times to remove the dissolved products. It was then air dried for about a week. The dried biomass was stored in clear Ziploc bags for later use. The average moisture content of the treated sugarcane trash was 0.070 g of water/g of wet sugarcane trash and the average ash content was 0.340 g of ash/g of sugarcane trash. The lime-treated sugarcane trash consisted of 0.660 g VS/g of dry sugarcane trash (0.536 g of carbohydrates/g of dry sugarcane trash and 0.124 g of lignin/g of dry sugarcane trash).

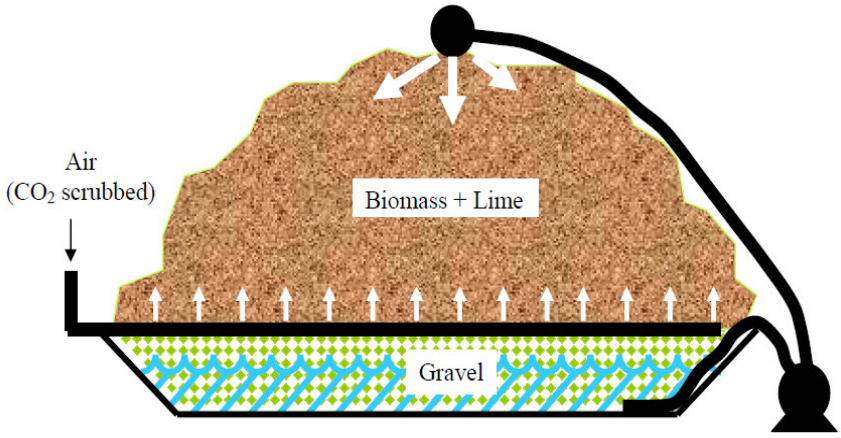


Figure 2-1. Cross-sectional view of air-lime pretreatment.

Figure 2-2. Photograph of biomass/lime pile formation in storage bin.

Figure 2-3. Photograph showing network of water sprayers on top of pile.

Figure 2-4. Photograph of lime slurry container.

2.2.2 Long-term Submerged Pretreatment

Long-term, submerged, air-lime pretreatment was performed in a large cylindrical vessel, as shown in Figure 2-5. This vessel can handle larger amounts of biomass similar to pile pretreatment in the tub. The procedure follows: a measured amount of biomass was mixed well with 0.4 g $Ca(OH)_2$/g dry biomass. Sufficient amount of distilled water was then added to the vessel to submerge the biomass/lime mixture. Air circulation through the mixture was achieved using an air hose connected to the main air line and placed below the base sieve plate in the cylinder. The air was scrubbed to remove carbon dioxide in a lime slurry vessel. Air flow was controlled through a ball valve located directly above the pretreatment vessel. The temperature controller maintained the pretreatment mixture at 50°C. Heating was achieved by using a heating element in a water tank. A thermocouple placed in the tank measured the temperature. The hot process water in the tank was pumped to the top of the pretreatment vessel. The cylindrical vessel served as a jacketed heat exchanger and the hot process water exited the vessel at the base of the cylindrical vessel. Both distilled water and process water had to be regularly added to maintain the liquid level in the pretreatment vessel and to replace the evaporated water in the hot water tank, respectively. The average moisture content of the treated sugarcane trash was 0.080 g of water/g of wet sugarcane trash and the average ash content was 0.395 g of ash/g of sugarcane trash. The lime-treated sugarcane trash consisted of 0.605 g VS/g of dry sugarcane trash (0.470 g of carbohydrates/g of dry sugarcane trash and 0.135 g of lignin/g of dry sugarcane trash).

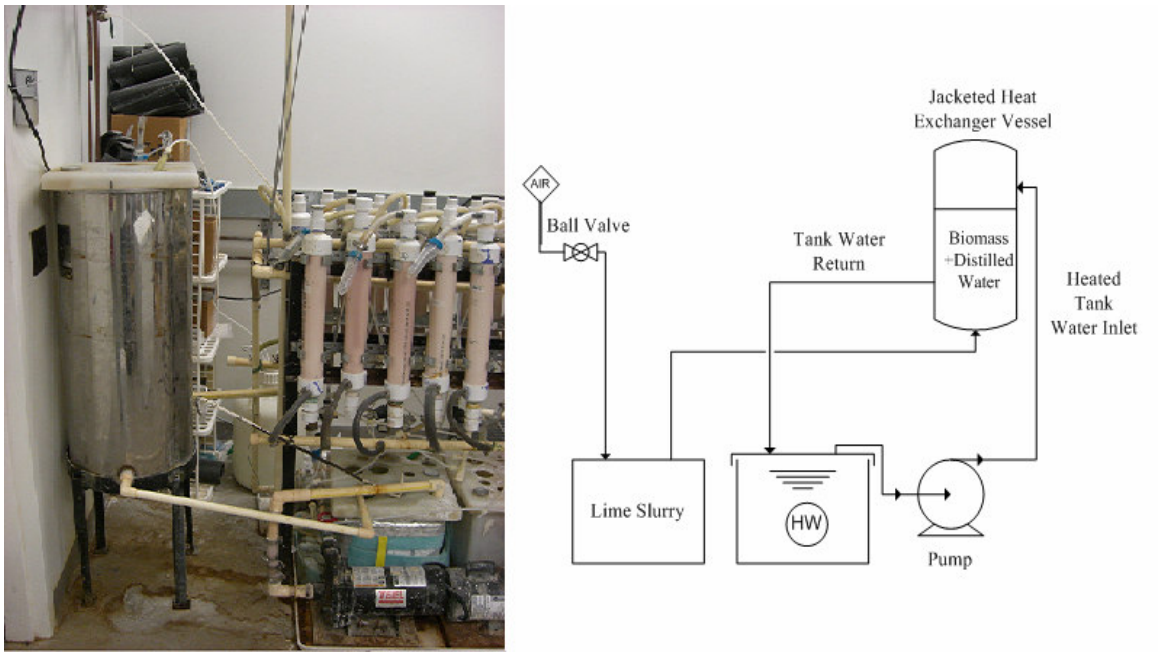


Figure 2-5. Photograph and schematic of submerged pretreatment apparatus.

2.2.3 Short-Term Pretreatments

Short-term oxidative lime pretreatment (Appendix C) can be performed under a variety of temperature and pressure conditions. Based on previous research work on sugarcane bagasse performed by Dr. Holtzapple's group, a temperature of 110°C and pressure of 0.791 MPa (100 psig) was selected. Six 304 stainless steel pipe nipples with 145-mL volume were used as the reactors for the short-term oxidative pretreatment. Because it was desirable to maintain a constant oxygen pressure, the reactors were sealed tight on both ends with Teflon tape and 3.81 cm (1.5-in) 304 stainless steel screw caps. The loadings for the reactor are 8 g of raw dry biomass, 15 mL of distilled water/g dry biomass, and 0.4 g $Ca(OH)_2$/g dry biomass. The oven was initially preheated to the

desired temperature before loading the reactor manifold assembly into the oven. The manifold was connected to the oxygen line via a 0.64 cm (0.25-in) flexible stainless steel hose 100 cm in length. The swinging arm operated by a motor attached to the back of the oven ensured that the biomass/lime slurry was well mixed during pretreatment. Figure 2-6 shows the experimental apparatus for the pretreatments.

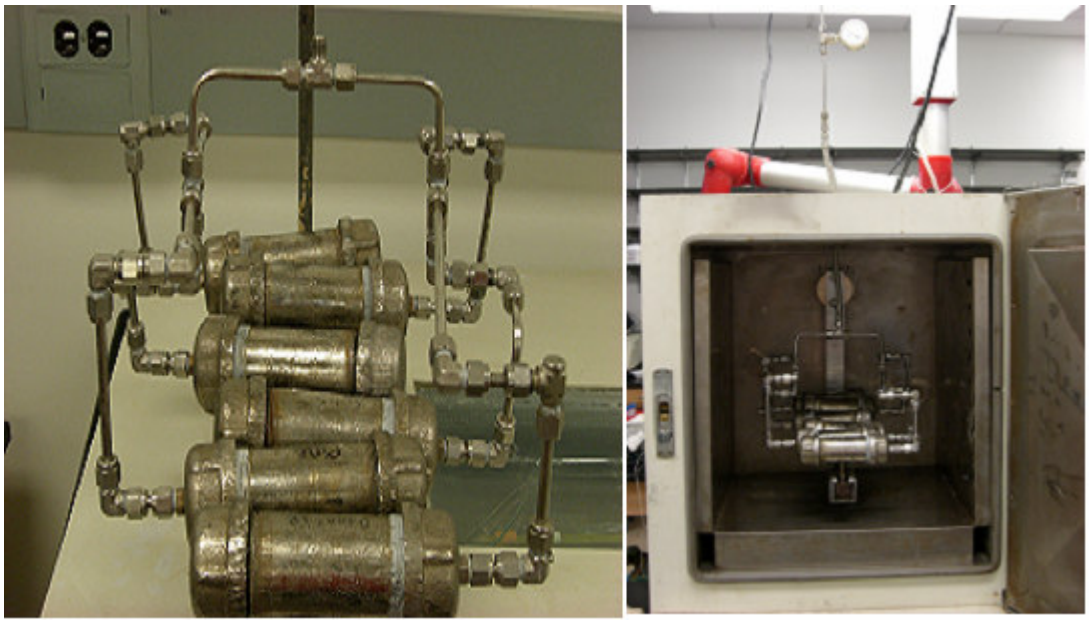


Figure 2-6. Photographs showing reactor assembly and operation in oven.

All pretreatments were performed at the same conditions because these conditions had already been determined to be optimum for a similar substrate. The main disadvantage with this procedure was the small amount of biomass which could be pretreated during one cycle. An excess of $Ca(OH)_2$ is usually added to eliminate one of the variables. Typically back titration was used to find the amount of lime consumed.

Because this pretreatment method involves high temperatures and pressures, extreme care and precaution were taken when operating the equipment and proper safety equipment was worn.

Two different neutralization procedures were used for the pretreated biomass. One involved neutralizing with acetic acid and washing with distilled water. Washing was done using a mix-stir-centrifuge cycle. The centrifuge cake was eventually air dried in the hood for 2–3 days and stored in air-tight Ziploc bags for later use.

Mix-Stir-Centrifuge cycle consisted of the following steps:

1) Adding sample in 1-L centrifuge bottle and filling with distilled water
2) Neutralizing to below pH 7.0 by adding drops of acetic acid while stirring
3) Centrifuging at 4000 rpm for 25 minutes (Beckman floor Centrifuge Model#J-6B)
4) Checking pH and noting color of liquid
5) Pouring away liquid and adding fresh distilled water
6) Adjusting pH if necessary and re-centrifuging for 25 min
7) Repeating Steps 4–6 until pH and color of liquid do not change for a minimum of six cycles.

The second neutralization procedure involved bubbling carbon dioxide through the pretreated biomass sample to decrease the pH below 7.0. This was also air dried in the hood and stored for later use. The dried pretreated samples were labeled as Short-Term Wash (STW) and Short-Term No-Wash (STNW).

2.2.4 Ball Milling

Ball milling decrystallizes biomass and increases its digestibility. It grinds the biomass to a powder. Porcelain jars (300-mL) were charged with 0.375-in zirconia grinding medium (U.S. Stoneware, East Palestine, OH). Biomass was placed in the jar to fill the void volume between the zirconia beads. The ratio of grinding medium to biomass was 43 g zirconia/g dry biomass. The jars were then placed between the rollers and rotated at 68 rpm for 3 days. After 3 days, the biomass was collected from the jars and labeled for later use. Figure 2-7 shows the ball milling apparatus.

Figure 2-7. Photograph showing porcelain jars and zirconia beads on the left. Ball milling rollers are shown on the right.

2.3 Liquid Media

The liquid medium used in the fermentations was deoxygenated water (Appendix D), which was prepared by boiling under a nitrogen purge for 10 min. The medium was

then allowed to cool to room temperate while being capped. To remove the remaining oxygen, 0.275 g/L of cysteine hydrochloride and 0.275 g/L of sodium sulfide were added under a nitrogen purge. The solution was well stirred and transferred to glass storage bottles under a nitrogen purge. Both cysteine hydrochloride and sodium sulfide are oxygen reducers.

2.4 Inoculum

Marine inoculum used in this research was obtained from sandy beach sediments at 8th Mile, East Beach, 51st St, and 9th St from Galveston, TX. The sediments were collected from 0.5-m holes and placed in 1-L centrifuge bottles, which were half filled with the deoxygenated liquid medium. Marine inoculum has a high salinity content which enables the microorganisms to adapt well to the carboxylate salts products under the buffered fermentations.

2.5 Inhibitor

Methanogens produce methane under anaerobic conditions through methanogenesis (Peters and Conrad, 1995). Because the MixAlco process requires anaerobic fermentation conditions, methanogenesis is a potential source for concern. Methanogenesis is the natural final stage of anaerobic fermentation. Carboxylic acids produced are intermediates in the fermentation of biomass to methane (Datta, 1981; Fukuzaki et al., 1990). Both carbon dioxide and acetic acid can serve as the terminal electron acceptor as shown by Equations 2-1 and 2-2. Methane formation can be

inhibited by the presence of ammonium ions or using methane analog inhibitors, such as iodoform or bromoform. This inhibition eliminates a potential hydrogen sink and instead is used to produce higher carboxylic acids, such as propionate and butyrate (Russell and Martin, 1984; Latham and Wolin, 1977).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (2\text{-}1)$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (2\text{-}2)$$

Ammonium bicarbonate has been determined to be a weak methane inhibitor (Fu, 2007). Iodoform (CHI_3), which is a strong methane inhibitor, was used in the anaerobic fermentations. The concentration of the iodoform solution was 20 g/L. It was prepared by dissolving 2 g of iodoform in 100 mL of ethanol. The iodoform solution was kept in a tinted bottle and capped immediately after use. This was done because the solution is oxygen and light sensitive. The bottle was stored in the refrigerator.

2.6 Buffer (pH Control)

In the MixAlco process, as microorganisms digest the biomass and convert it into a mixture of carboxylic acids, the pH must be controlled. Because of the inhibitory effects of carboxylic acids, a buffer is added to control the pH so that the microorganisms will not be hindered by the low pH of the acids. Traditionally calcium carbonate ($CaCO_3$) has been the preferred choice of buffer. It is relatively inexpensive and can be easily converted to lime for use in pretreatments. However, recent research in Dr. Holtzapple's group has shown that ammonium bicarbonate (NH_4HCO_3) buffered

fermentations produce an average increase of 50–60% in carboxylic acid concentrations compared to calcium carbonate buffered fermentations (Fu, 2007; Agbogbo 2005). This is because the pH for ammonium bicarbonate fermentations can be controlled around 6.5 to 7.5, with the optimum being at 7.0. Most microorganisms that convert biomass to carboxylic acids prefer a near-neutral environment. Ammonium bicarbonate fermentations also produce higher acetate contents (~80%), whereas calcium carbonate fermentations are lower (~60%) Agbogbo (2005). This is useful when the desired product is ethanol in the MixAlco process.

Ammonium bicarbonate is a white crystalline solid with a faint odor of ammonia and is stable at ambient temperature, but decomposes upon heating to 60°C. It melts at 107.5°C with very rapid heating (Patnaik, 2002). Ammonia nitrogen is an essential nutrient for anaerobic microbes (Katagiri and Nakamura 2002). Ammonium salts can inhibit methanogenesis as well (Kayhanian, 1999; Parkin and Speece, 1982).

The pH was measured and monitored using an ORION portable pH/temperature meter (Model# 230A). The Triode^{TM} 3-in-1 combination pH/ATC electrode 58819-91 with BNC connector allowed the pH meter to rapidly measure pH in the anaerobic fermentation system.

2.7 Fermentors

The rotary fermentors (Figures 2-8 and 2-9) were made from Beckman 1-L polypropylene centrifuge bottles (98 × 169 mm), Nalgene brand NNI 3120-1010. A size-11 rubber stopper was used to close the bottles with a hole drilled in the middle. A glass tube with a rubber septum for gas sampling and venting was inserted through the hole. The rubber septum was replaced when there was a visible hole because of frequent puncturing to vent the produced gas. Two 0.25-in stainless steel tubes with their ends welded shut were inserted into holes in the stopper. These stainless steel bars were used as stirrers to mix the components of the fermentor. The fermentor could not withstand pressures greater than 2 atm, thus the gas was vented frequently to prevent pressure buildup. The fermentor bottles were placed in a Wheaton Modular Cell Production Roller Apparatus (Model III). This apparatus was placed on rollers in an incubator (Figure 2-10), which rotated horizontally at 2 rpm. The incubator was maintained at 55°C, which is characteristic of thermophilic fermentations.

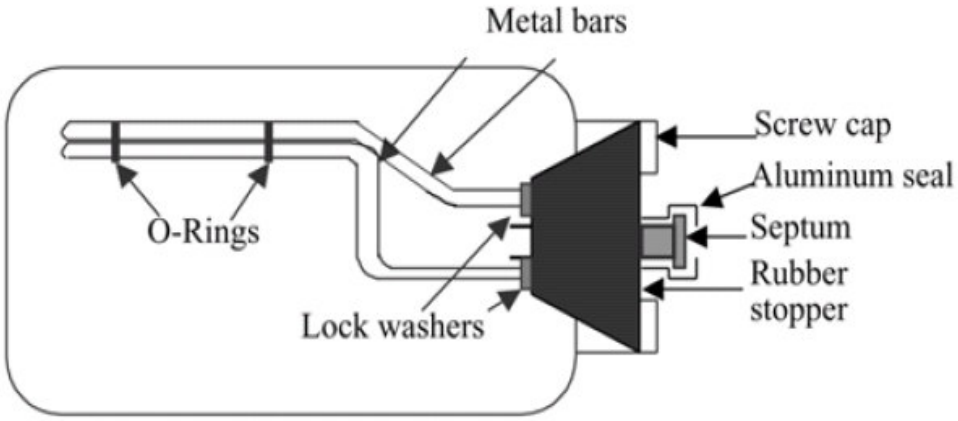


Figure 2-8. Components of assembled fermentor.

Figure 2-9. Photograph of rotary fermentors.

Figure 2-10. Photograph of the fermentation incubator.

2.8 Anaerobic Fermentations Procedure

Batch fermentation and countercurrent fermentations were performed in this research. Batch fermentations were usually performed for about 20–30 days or until the acid concentration stopped increasing. Countercurrent fermentations were performed until the system reached a steady state, which took about 2–4 months. Usually 3–4 weeks of data were collected from countercurrent fermentations once the system reached steady state.

2.8.1 Batch Experiments

Batch experiments involved loading the desired amount of substrates and nutrients to the fermentors in an initial charge. Subsequently, during sample collection, only iodoform was added to inhibit methanogenesis. Batch experiments in the laboratory were initiated by adding biomass and chicken manure in 80/20 ratio to 1-L centrifuge bottles. Deoxygenated water and fresh marine inoculum were added to achieve the desired concentration. Ammonium bicarbonate buffer was added if the pH was below 7.0. Iodoform was also added to inhibit methanogensis. The fermentors were then capped and placed in the incubator where they rotated at 2 rpm. The incubator was maintained at 55°C (thermophilic condition). During the preparation process, nitrogen purge was maintained in the fermentors to ensure an anaerobic environment.

2.8.2 Countercurrent Experiments

Countercurrent experiments (Figure 2-11) were initiated as batch experiments for a period of two weeks. This allowed for the culture to be established. After two weeks, a constant biomass loading was used for each fermentor train during every transfer. Solid and liquid transfers were performed every two days using the single-centrifuge transfer procedure (Appendix E). Gas production in the fermentors was measured and recorded. The fermentors were opened under nitrogen purge and capped with centrifuge bottle caps. The fermentors were then centrifuged for 25 min to separate the solids from the liquids. The product liquid was decanted into a measuring cylinder. The liquid volume was recorded and a 3-mL product liquid sample was withdrawn only from Fermentor 1 (F1). The remaining liquid was poured into a collection bottle for volatile solids analysis

later. For the other bottles, the liquid volume was recorded and transferred upstream only (e.g., F3 to F2; F4 to F3). A fixed amount of fresh liquid medium was added to the last fermentor (F4) in each train. A predetermined wet cake weight was maintained in all fermentors. Solids were transferred from F1 to F4 to maintain the cake weight. Excess solids from F4 were removed and stored in a collection bottle for later volatile solids analysis. Transfers were performed under nitrogen purge at all times to maintain anaerobic conditions. Once the transfer was completed, pH adjustments were done and iodoform was added. The fermentors were closed and placed back in the incubators. Steady state was reached when consistent acid concentration was produced for 10 transfers in a row.

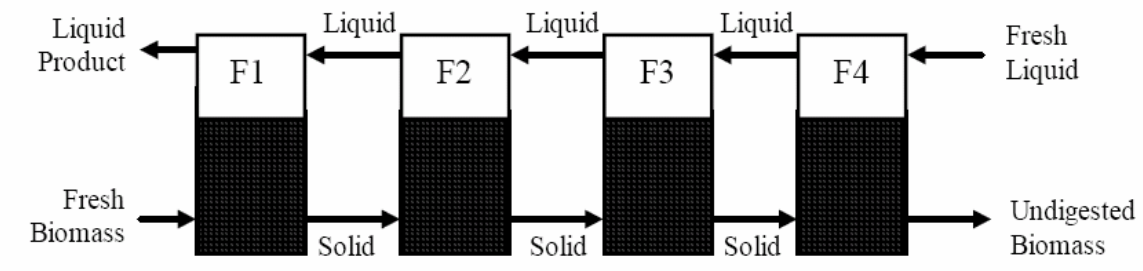


Figure 2-11. Typical flow diagram for countercurrent transfers (Source: Fu, 2007).

2.9 Mass Balance Closure for Countercurrent Experiments

Biomass consists mainly of volatile solids (VS) and ash (Figure 2-12). Lignin is the main unreactive portion of the volatile solids whereas the ash component of biomass is totally unreactive. When biomass is digested, the volatile solids (except for lignin) are converted to liquid and gaseous products. There is also a solid residue of undigested

volatile solids and ash. The gaseous products consist of mainly methane and carbon dioxide. Because an inhibitor is added, the methane content is minimal (typically less than 0.5%). The liquid products consist of carboxylate salts, extracellular protein, and energy storage polysaccharides (Ross, 1998).

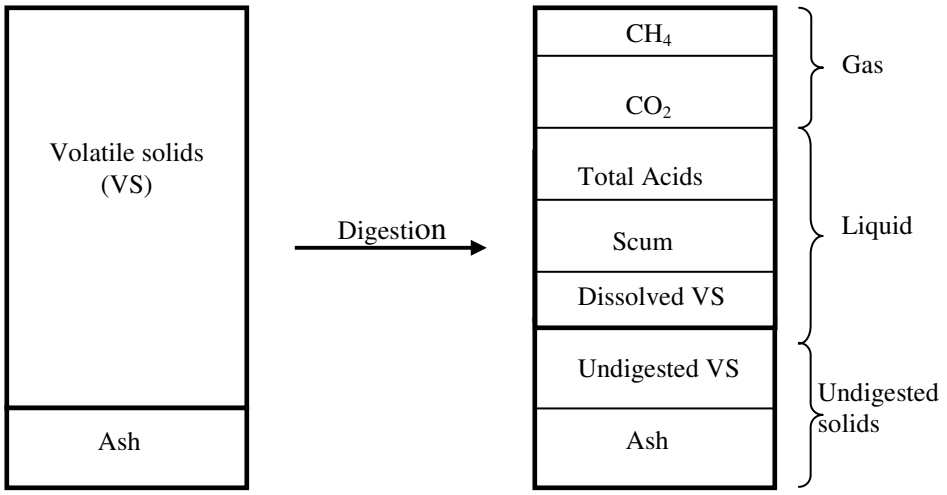


Figure 2-12. Digestion of biomass.

Mass balance for countercurrent experiments was performed for the steady-state period. Mass balance closure is represented as the ratio of mass of products and mass of reactants, including the water of hydrolysis. In theory, there should be 100% closure. Deviations usually result from inaccurate gas volume measurements and human errors involved in transfer procedures. Only biotic carbon dioxide is used in the calculations. Abiotic carbon dioxide is produced when the buffer neutralizes the carboxylic acid. The amount of biotic CO_2 is calculated by subtracting the abiotic CO_2 from the total amount

of CO_2 produced. Abiotic CO_2 can be calculated either from Equations 2-3 or 2-4, depending on the buffer being used. For ammonium bicarbonate buffer, each mole of carboxylic acid neutralized produces one mole of abiotic CO_2. The biotic carbon dioxide is usually very small because mixed acid fermentation produces little or no carbon dioxide as the reducing power of glucose can be converted entirely to acetic acid as shown by Equation 2-5.

$$2\ CH_3(CH_2)_xCOOH + CaCO_3 \rightarrow Ca(CH_3(CH_2)_xCOO)_2 + H_2O + CO_2 \qquad (2\text{-}3)$$

$$CH_3(CH_2)_xCOOH + NH_4HCO_3 \rightarrow NH_4CH_3(CH_2)_xCOO + H_2O + CO_2 \qquad (2\text{-}4)$$

where $x = 0,1,2,3,4,5$

$$C_6H_{12}0_6(\text{glucose}) \rightarrow 3\ CH_3COOH\ (\text{acetic acid}) \qquad (2\text{-}5)$$

The mass balance equations follow:

$$\text{Mass in} + \text{water of hydrolysis} = \text{mass out} \qquad (2\text{-}6)$$

$$\text{VS in} + \text{water of hydrolysis} = \text{VS out} \qquad (2\text{-}7)$$

$$\text{VS in} + \text{water of hydrolysis} = \text{carboxylic acid produced} + \text{biotic } CO_2 + CH_4 + \text{dissolved VS} + \text{undigested VS} \qquad (2\text{-}8)$$

To facilitate calculation of water of hydrolysis, Ross (1998) assumed biomass could be represented as cellulose, which has a molecular weight of 162 g/mole. When

cellulose is hydrolyzed, it gains a molecule of water per monomer. Thus, water of hydrolysis can be calculated as:

$$\text{Water of hydrolysis (g)} = \text{VS digested (g)} \times \frac{18\,\text{g/mol}}{162\,\text{g/mol}} \tag{2-9}$$

Eventually mass balance closure is calculated as:

$$\text{Closure} = \frac{\text{Undigested VS+dissolved VS+Acid Produced+}CO_2\text{+}CH_4}{\text{VS in + Water of Hydrolysis}} \tag{2-10}$$

2.10 Fermentation Operation and Performance Parameters

The operating parameters for countercurrent fermentations are liquid residence time (LRT) and volatile solids loading rate (VSLR). Liquid residence time determines how long the liquid remains in the system. Long liquid residence times allow for higher product concentrations, but also require large reactor volumes (Holtzapple et al., 1999). Liquid residence time is calculated as:

$$\text{LRT} = \frac{\text{TLV}}{\text{Q}} \tag{2-11}$$

where,

Total Liquid Volume (TLV) = Sum of liquid volume in all four fermentors F1–F4

Q = Amount of liquid removed per day from F1 (L/day)

TLV can be calculated as:

$$\text{TLV} = \sum_i (\overline{K}_i \cdot w_i + \overline{F}_i) \tag{2-12}$$

where,

$\overline{K}_i$ = Average wet mass of solid cake in Fermentor *i* (g)

w_i = Liquid fraction of solid cake in Fermentor *i* (L liquid/g wet cake)

$\overline{F}_i$ = Average volume of free liquid in Fermentor *i* (L liquid)

The VSLR represents the rate at which substrates are added to the fermentation system. A low VSLR increases the solid residence time, which is a measurement of how long the solids remain in the system. Longer solids residence time increases product yields. VSLR can be calculated as:

$$\text{VSLR} = \frac{\text{VS fed /day}}{\text{TLV}} \tag{2-13}$$

The performance parameters are used for both countercurrent and batch experiments to quantify the results and performance of these fermentations. The parameters allow for comparison between similar fermentations.

$$\text{Yield} = \frac{\text{Total acids produced}}{\text{VS fed}} \tag{2-14}$$

$$\text{Conversion} = \frac{\text{VS digested}}{\text{VS fed}} \tag{2-15}$$

$$\text{Total acid selectivity} = \frac{\text{Total acids produced}}{\text{VS digested}} \tag{2-16}$$

$$\text{Total acid productivity} = \frac{\text{Total acids produced}}{\text{Total liquid volume in all fermentors} \cdot \text{Time}} \tag{2-17}$$

2.11 Analytical Methods

This section describes the analytical methods used in the laboratory. It describes the equipment and methods used to determine gas volume, gas composition, carboxylic acid concentration, acid composition, volatile solids content, and lignin composition of biomass.

2.11.1 Gas Volume Measurement

The volume of gas produced in the fermentors is measured using water displacement (Figure 2-13). The device consists of inverted graduated glass cylinders filled with 30% $CaCl_2$ solution. The $CaCl_2$ minimized microbial growth and prevented CO_2 adsorption due to the acidic pH. The fermentors were cooled down to room temperature before measuring gas production. Initially, suction raised the liquid level to the top of the column. A hypodermic needle was inserted through the fermentor septum and the released gases displaced the liquid in the glass cylinder until the pressure in the fermentor equaled the pressure in the headspace of the cylinder. The internal diameter of the glass cylinders was 5.2 cm. The recorded displacement length (L) in cm was converted to gas volume (V) by the following equation: $V(mL)=21\ cm^2 \times L\ (cm)$.

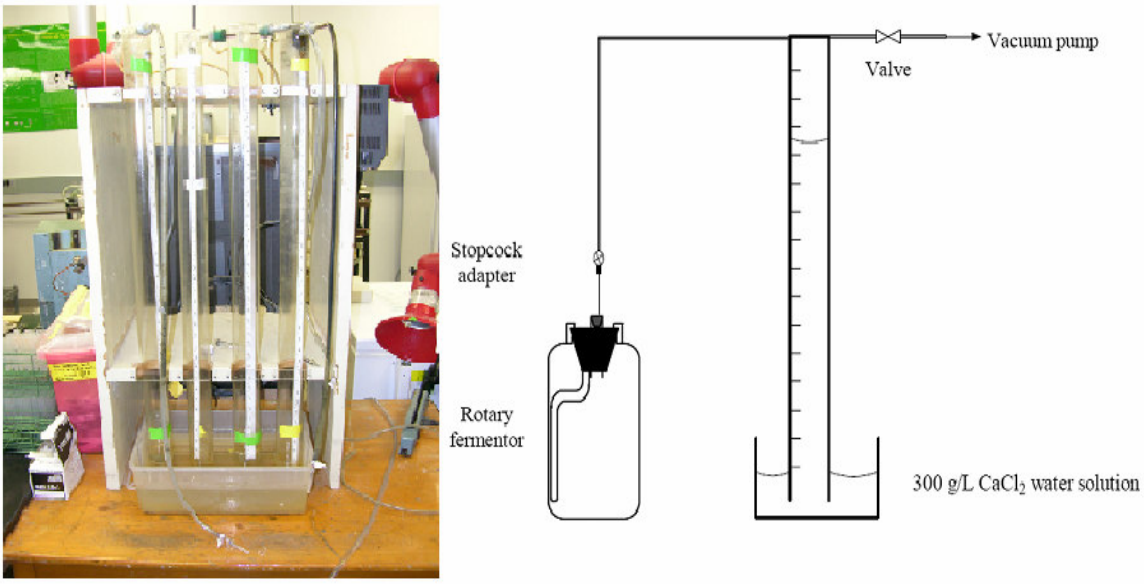


Figure 2-13. Photograph and diagram of gas volume measurement device (adapted from Fu, 2007).

2.11.2 Gas Content Measurement

A gas chromatograph (Agilent 6890 series, Figure 2-14) with a thermal conductivity detector (TCD) was used to determine the methane and carbon dioxide composition of the fermentation gas. Gas samples were withdrawn through the rubber septum in the fermentors using a 5-mL syringe. Gas samples for analysis were usually collected before measuring the gas volume. The volume of gas withdrawn was then added to the total volume of gas produced. A standard gas mixture of carbon dioxide (29.99 mol%), methane (10.06 mol%), and the remainder nitrogen was routinely used to calibrate the gas chromatograph.

2.11.3 Liquid Sample Analysis

A 3-mL liquid sample was collected for carboxylic acid concentration and composition measurement. The samples were stored in freezer bags until a sufficient amount of samples were collected for analysis in the gas chromatograph. The samples were thawed and well mixed before being prepared for analysis. Carboxylic acid concentration was measured using an Agilent 6890 series (Figure 2-14) gas chromatograph with capillary column (J&W Scientific, model DB-FFAP). The gas chromatograph was equipped with a flame ionization detector (FID) and a 7683 series injector. The samples were acidified with 3-M phosphoric acid and mixed with 1.162 g/L of internal standard solution (ISTD). The internal standard used was 4-methyl-*n*-valeric acid. A standard carboxylic acids mix (Matreya Inc., catalog #1075) was injected prior to injecting the samples in the sequence for calibration purposes. The oven temperature in the GC is increased from 50 °C to 200 °C at 20 °C/min and held for an additional 1 minute at 200 °C. More details on the liquid sample preparation procedure for analysis are described in Appendix F.

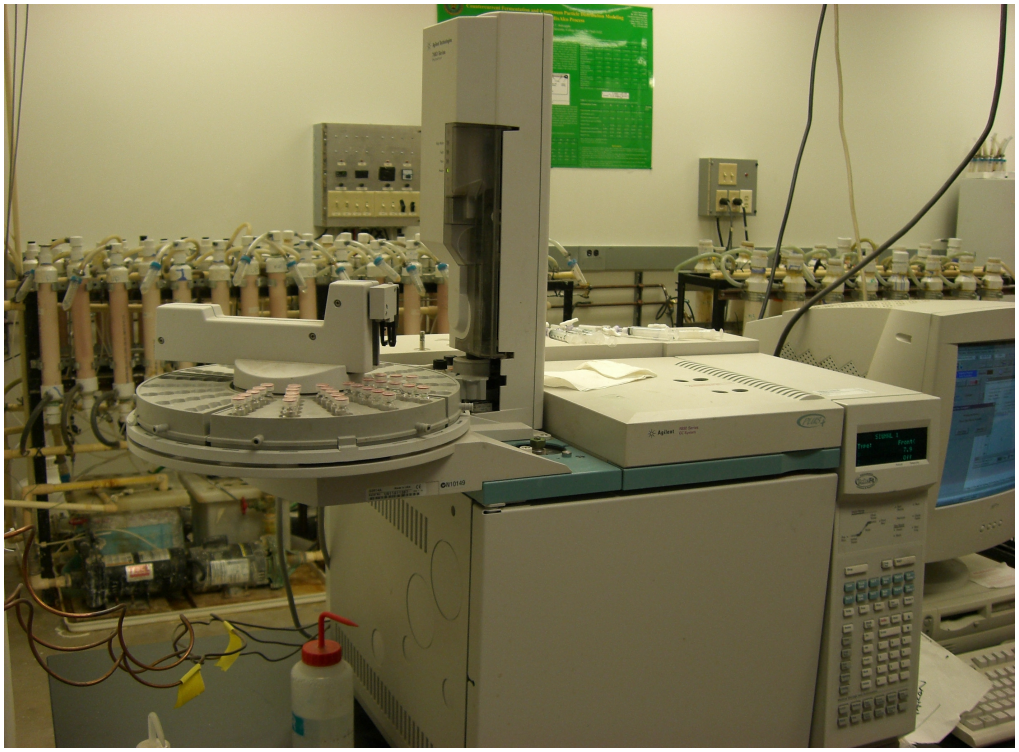


Figure 2-14. Photograph of Agilent 6890 series gas chromatograph.

2.11.4 Moisture Content and Volatile Solids Determination

The moisture content of the biomass substrates and chicken manure was determined by drying in an oven at 105°C overnight. The dried sample was then ashed in a muffle furnace at 550°C for 24 hours to determine the volatile solids content. For liquid samples, $Ca(OH)_2$ was added prior to drying to prevent un-neutralized carboxylic acids from being volatilized. Appendix G provides more detail on the volatile solids analysis procedure for liquid and solid samples.

2.11.5 Lignin Content Determination

Lignin content was determined in accordance with NREL Standard Procedure – Determination of Structural Carbohydrates and Lignin in Biomass (NREL, 2008b). The biomass sample was ground and sieved to particle sizes between 20 and 80 mesh. Concentrated sulfuric acid was then added to the samples and the samples are placed in a water bath at 30℃ for 1 h. Distilled water was added to the samples, which were then transferred to pressure vessels and placed in the autoclave for another hour at 121℃. After completing the autoclave cycle, the hyrolyzates were cooled to room temperature and filtered using vacuum filtration. The absorbance of the liquid filtrate was analyzed using a spectrophotometer to determine the acid-soluble lignin. The solids collected were ashed in a muffle furnace at 550℃ to determine the amount of acid-insoluble lignin in the sample. The total lignin is the sum of acid-soluble lignin and acid-insoluble lignin.

2.12 Continuum Particle Distribution Model (CPDM)

The CPDM method (Loescher, 1996) is very advantageous for predicting acid concentrations and conversions for various fermentation configurations and substrates. Parameters for the program are determined from batch experiments, thus the need for performing several countercurrent experiments to determine optimum operating conditions can be avoided. Countercurrent experiments can take several months to reach steady state and they have long residence times as well.

CPDM is used to quantify the kinetics of a reaction that occurs at the interface between solid and liquid phases. The concept of continuum particle is used to avoid the

difficulties of tracking the geometry of individual discrete particles. Loescher (1996) defined a continuum particle as a collection of biomass particles with two main properties: (1) A mass of one gram in the initial unreacted state and (2) a particle size distribution identical to the entire feedstock entering the fermentation. Ross (1998) modified Loescher's original definition and defined a continuum particle to have a volatile solids mass of one gram when entering the fermentation system. The particle concentration, S_0 (particles/L) is related to the particle distribution function as shown in Equation 2-18.

$$S_0 = \int_0^1 \hat{n}(x)dx \tag{2-18}$$

Equation 2-19 relates the total reaction rate (r) to the specific reaction rate ($\hat{r}$) as a function of particle conversion and product concentration, A. The specific reaction rate $\hat{r}(x,A)$ contains information about the reaction system and products and $\hat{n}(x)$ contains information about substrate concentrations and conversions.

$$r = \int_0^1 \hat{r}(x,A)\hat{n}(x)dx \tag{2-19}$$

For a batch reaction system, all the particles have the same conversions; therefore, $\hat{n}(x)$ will be zero everywhere except at x'.

$$n_0 = \int_0^1 \hat{n}(x)dx = \lim_{\varepsilon \to 0} \int_{x'-\varepsilon}^{x'+\varepsilon} \hat{n}(x)dx \tag{2-20}$$

The Dirac delta function can be used to represent the distribution function, as shown in Equation 2-21.

$$\hat{n}(x) = S_0 \delta(x - x') \tag{2-21}$$

Substituting Equation 2-21 into Equation 2-19 leads to the final form of the rate equation.

$$r = \int_0^1 \hat{r}(x, A)\hat{n}(x)dx = \int_0^1 \hat{r}(x, A)S_0\delta(x - x')dx = \hat{r}(x', A)S_0 \tag{2-22}$$

In conclusion, Equation 2-22 shows that the total reaction rate is related to the specific reaction rate $\hat{r}(x', A)$ by the initial particle concentration. Therefore, the specific reaction rate can be measured by performing batch experiments at various initial loading rates. CPDM parameters obtained from the batch experiments allow the user to determine optimum VSLR and LRT conditions to achieve desired product concentrations and conversions.

CHAPTER III

BATCH STUDIES

3.1 Purpose

Preliminary batch experiments were performed using sugarcane trash to evaluate its potential. Batch experiments offer a time-effective way to determine the suitability of sugarcane trash for the MixAlco process. The performance of sugarcane trash is compared with sugarcane bagasse and paper. Numerous studies have been previously conducted with bagasse (Fu, 2007; Thanakoses, 2002). Bagasse has already been determined to be an excellent feedstock for the MixAlco process.

3.2 Pretreatment of Sugarcane Trash

Lignocellulosic biomass, such as sugarcane trash, requires pretreatment to make the biomass more digestible and increase the carboxylic acid yields. The various pretreatments investigated in this research were presented in Chapter II. For the batch studies, long-term air-lime pile pretreated bagasse and sugarcane trash were used. Pretreated bagasse from a previous student's research (Fu, 2007) was used. Sugarcane trash was mixed with 0.4g $Ca(OH)_2$/g dry biomass and pretreated at 50°C for five weeks. Lignin degradation over the five weeks was tracked by removing solid samples from the system weekly. These samples were washed extensively to neutralize excess lime. The washed samples were air dried and ground using a coffee grinder to particle sizes between 25 and 80 mesh. These samples were analyzed for lignin according to NREL Standard Procedure – Determination of Structural Carbohydrates and Lignin in Biomass (NREL, 2008b). Figure 3-1 shows the lignin degradation.

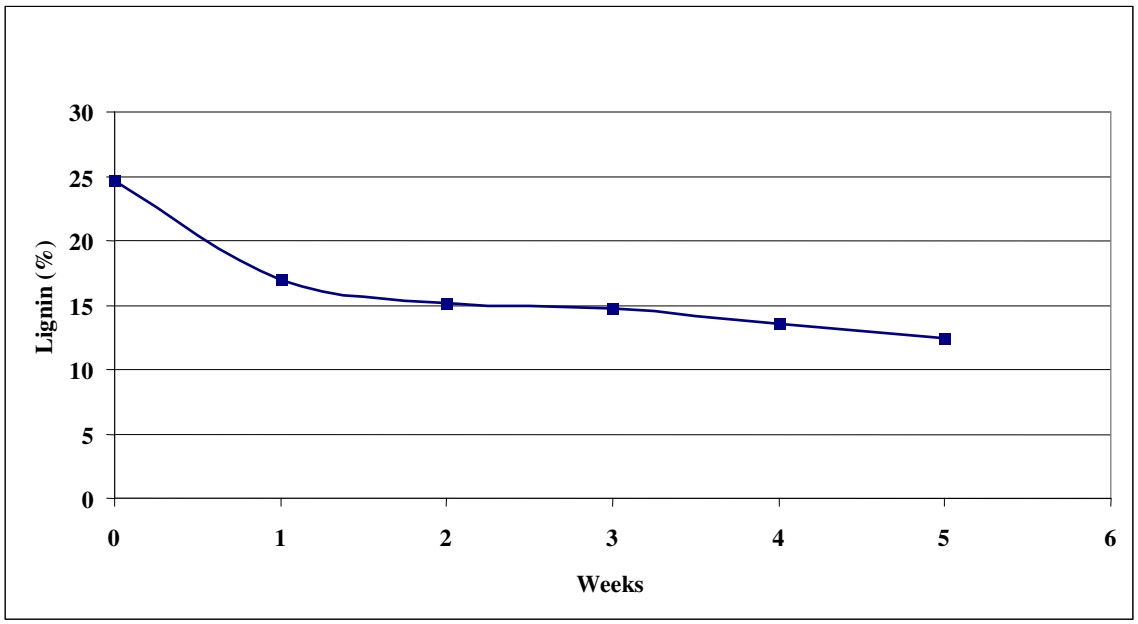


Figure 3-1. Lignin degradation during long-term air-lime pile pretreatment in sugarcane trash.

At the end of pretreatment, a representative sample was removed from the pile, thoroughly washed, neutralized with acetic acid, and air dried. This sample was later analyzed for sugars and lignin. The average glucan and xylan content was 49.6% and 20.9%, respectively. The average lignin content at the end of pretreatment was 12.4% (~ 50% reduction). This is a good approximation of the actual VS present at end of pretreatment, which is about 83%. The VS content of sugarcane trash reported on Table 2-1 was 66%. This number is significantly lower because carbon dioxide was used to neutralize the pretreated solids, which results in calcium carbonate production. Calcium carbonate is relatively insoluble and would become part of the ash added to fermentation. However, the excess calcium salts have very minimal effect on the performance of the fermentations (Fu, 2007). Ammonium bicarbonate is highly soluble

in water, whereas calcium carbonate is nearly insoluble. Thus, the produced carboxylic acids would react with the highly soluble ammonium bicarbonate buffer before reacting with the calcium carbonate. At a controlled pH of around 7.0, calcium carbonate consumption will be very unlikely and will remain as part of the unreactive ash. If acetic acid had been used to neutralize instead, the VS content would have been much higher because calcium acetate is easily washed away. Calcium carbonate complicates the analytical determination of the pretreatment yield. Smaller scale pretreatments in Erlenmeyer flasks were performed at similar conditions to determine the actual pretreatment yield. The solids could be recovered easily whereas for the pile it is impossible to account for all the solids due to loss in the gravel bed. The actual pretreatment yield for air-lime pretreatment at 50°C for 5 weeks was determined to be 0.70 g treated biomass/g untreated sugarcane trash. Thus, about 30% of the solids solubilized.

3.3 Batch Experiment Conditions

Anaerobic batch fermentations were performed for 20 days at 55°C. Three different substrate conditions were used for these batch experiments. To determine the reproducibility of the experiments, each set of condition was performed in triplicate:

1) A control of copier paper (80%) and chicken manure (20%) at 100 g/L.
2) Air-lime pretreated bagasse (80%) from previous student's research (Fu, 2007) and chicken manure (20%) at 100 g/L.
3) Air-lime pretreated sugarcane trash (80%) and chicken manure (20%) at 100 g/L.

Batch experiments were established by adding 32 g of dry substrate and 8 g of dry chicken manure to the 1-L centrifuge bottles. Deoxygenated water (350 mL), 50 mL of fresh inocula, and 120 μL of iodoform were also added to the bottle. Ammonium bicarbonate (2 g) was also added initially to each bottle. The fermentors were then purged with nitrogen and capped. They were placed in a roller incubator at 55°C. The gas was vented and liquid samples were collected every day. The pH was adjusted from 7.15 to 7.25 and 120 μL of iodoform was added every other day. Each fermentor was then purged, capped, and returned to the incubator. The gas produced was analyzed every few days for methane production.

3.4 Results

Batch experiments for the three different substrates were performed for 20 days under thermophilic (55°C) and anaerobic conditions. Figure 3-2 shows the average total acid concentration obtained for the three different substrates. The *y* error bars represent one standard deviation of the data. The overlapping error bars shows that there is very little difference between sugarcane trash and bagasse. Figure 3-3 shows that the average acetate content in the batch experiments for all the three substrates was 85–90%. This is very beneficial if the desired end-product of the MixAlco process is ethanol.

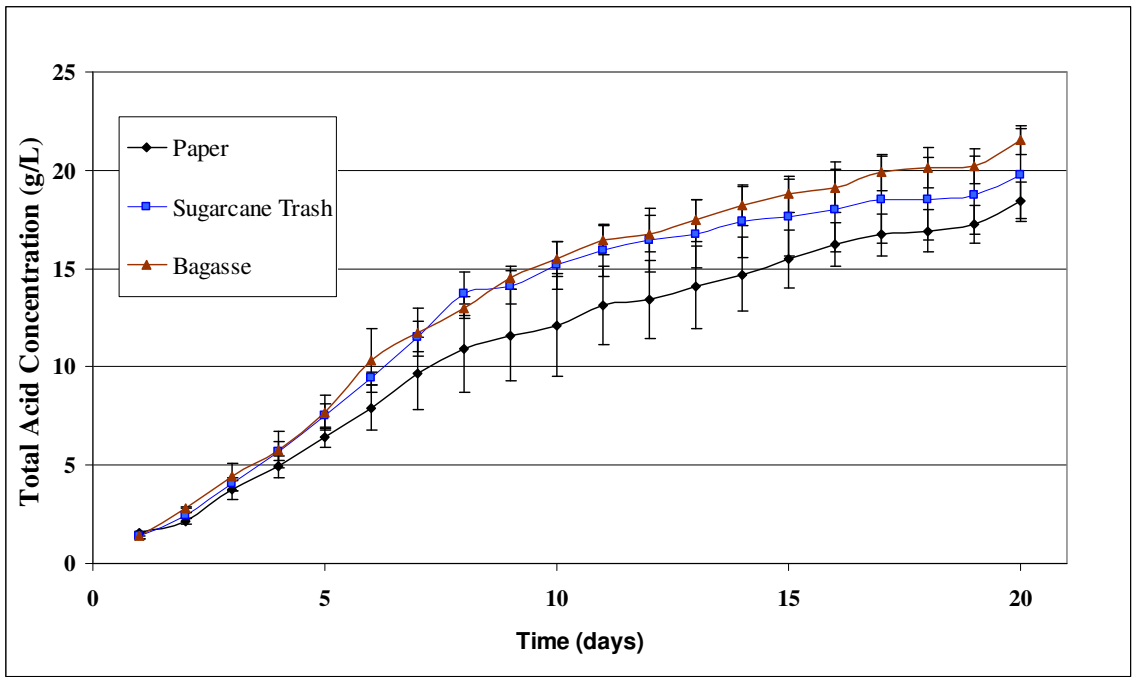


Figure 3-2. Carboxylic acid concentration for the batch experiments.

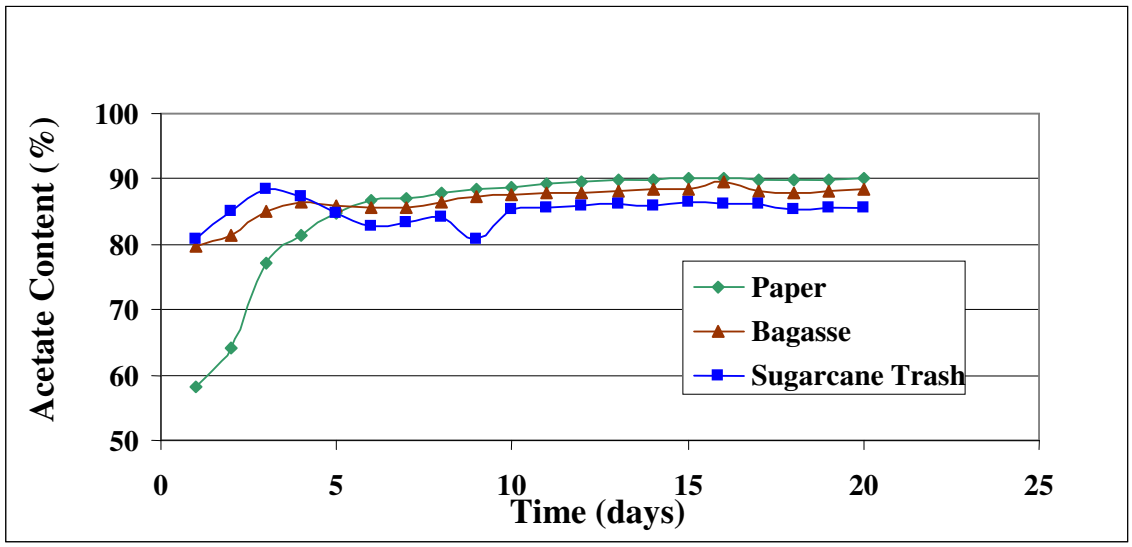


Figure 3-3. Average acetate content in the batch fermentations.

Table 3-1 shows the final acid concentration obtained from the batch experiments at the end and the weight fractions of the acids produced. It also has other key parameters, such as conversion, selectivity, and yield. Overall yield, conversion, and selectivity were determined using Equations 3-1 to 3-3.

$$\text{Overall Yield} = \text{Pretreatment Yield}\left(\frac{\text{g VS treated biomass}}{\text{g VS untreated biomass}}\right)\times \text{Fermentation Yield}\left(\frac{\text{g acids produced}}{\text{g VS treated biomass}}\right) \tag{3-1}$$

$$\text{Overall Selectivity}\left(\frac{\text{total acids produced}}{\text{g VS untreated- g VS undigested}}\right) = \frac{\text{Fermentation Selectivity}\times\text{Fermentation Conversion}\times\text{Pretreatment Yield}}{\text{Overall Conversion}} \tag{3-2}$$

$$\text{Overall Conversion}\left(\frac{\text{g VS untreated-g VS undigested}}{\text{g VS untreated}}\right) = \text{Pretreatment Conversion}\left(\frac{\text{g VS untreated-g VS treated}}{\text{g VS untreated}}\right)+ \text{Fermentation Conversion}\left(\frac{\text{g VS treated-g VS undigested}}{\text{g VS treated}}\right)\times \text{Pretreatment Yield}\left(\frac{\text{g VS treated}}{\text{g VS untreated}}\right) \tag{3-3}$$

Table 3-1. Results for batch fermentation with ammonium bicarbonate buffer after 20 days

	Sugarcane Trash	Bagasse	Paper
Total carboxylic acid concentration (g/L)	19.7 ± 2.35	21.6 ±0.728	18.4 ± 0.920
Acetic acid (wt%)	85.7 ± 4.62	88.4± 3.24	90.0 ± 0.856
Propionic acid (wt%)	1.99 ± 0.288	2.06 ± 0.395	1.58 ± 0.195
Butyric acid (wt%)	11.1 ± 4.08	8.77 ± 2.71	7.40 ± 0.837
Valeric acid (wt%)	1.23 ± 0.288	0.751 ± 0.140	0.993 ± 0.151
Caproic acid (wt%)	0	0	0
Heptanoic acid (wt%)	0	0	0
Fermentation conversion (g VS digested/g VS fed)	0.70 ± 0.03	0.64 ± 0.03	0.42 ±0.04
Fermentation yield (g total acid/g VS fed)	0.31 ± 0.04	0.27 ± 0.01	0.23 ± 0.01
Fermentation selectivity (g total acids/g VS digested)	0.45 ± 0.07	0.42 ± 0.02	0.55 ± 0.04

Note: Errors are ± 1 standard deviation

3.5 Conclusions

The batch studies show that batch fermentations with sugarcane trash produce similar carboxylic acid concentrations as bagasse. Sugarcane trash had the highest average yield (0.31 g total acid/g VS fed) and highest average conversion (0.70 g VS digested/g VS fed) among the three substrates compared. Sugarcane trash (19.7 g/L) produced more acids than the control paper (18.4 g/L). Both sugarcane trash and bagasse have similar selectivity (~ 0.45 g total acids/g VS digested). Sugarcane trash is a very promising alternative source of lignocellulosic biomass to be used in the MixAlco

process. In an industrial setting, the fermentation could be run in a countercurrent fashion with high VS concentrations to achieve high conversions and product concentrations. The next few chapters attempt to prove this hypothesis.

CHAPTER IV

SUGARCANE TRASH COUNTERCURRENT FERMENTATION

4.1 Purpose

Countercurrent fermentations mitigate the inhibitory effects associated with accumulating carboxylate salts by adding fresh liquid to the most digested biomass and continually removing the concentrated product liquid. This allows for high product concentrations and conversions. In this study, countercurrent fermentations using 80% pretreated sugarcane trash and 20% chicken manure are performed at various VSLR and LRT. CPDM is used to create a "map" that allows for the determination of acid concentration and conversions for desired combinations of VSLR and LRT. The accuracy of the CPDM prediction is determined by comparing the predicted values with the experimental values.

4.2 Countercurrent Fermentation Conditions

Countercurrent fermentations were performed at thermophilic conditions (55°C). Anaerobic conditions were maintained by having a nitrogen purge in the bottles whenever they were open. A series of three fermentor trains with different solid loading rates were established. Initially, the fermentors were operated in batch mode for a period of two weeks, which allows for the culture to be established. The batch fermentations were established by adding 32 g of dry substrate and 8 g of dry chicken manure to the 1-L centrifuge bottles. Ammonium bicarbonate (2 g) was added initially to each bottle. Deoxygenated water (350 mL), fresh inocula (50 mL), and iodoform (120 μL) were also

added to the bottle. Samples from the batch experiments were collected every 2 days for 2 weeks. pH adjustments and iodoform addition were maintained during these two weeks as well. Transfer procedures were initiated after two weeks of operation in batch mode. Solid and liquid transfers were performed every two days using the single-centrifuge procedure. Table 4-1 shows the operating parameters for the three fermentor trains. On each transfer, a fixed amount of solids as shown on Table 4-1 and 80 μL of iodoform were added to F1. Iodoform solution (40 μL) was added to F2, F3, and F4. Fresh deoxygenated water (100 mL) was added to F4 on each transfer. The pH in each fermentor was adjusted to between 7.00 and 7.25. A target goal weight of 300 g for the solid cake in the fermentors was used. To more rapidly achieve the target goal weight, increased solid loadings were used in the first month. However, steady-state data were only collected after about three months of operation. Figures 4-1 to 4-3 show the total acid concentration profile as the trains approached steady state. Steady-state (±5 g/L total acid concentration) fermentation data were used to determine acid productivity, yield, selectivity, conversion, CH_4 productivity, and biotic CO_2 productivity. The total free liquid for the fermentations was determined by Equation 2-12, which includes the free liquid obtained after centrifuging as well as the liquid content in the wet solid cake.

Table 4-1. Operating parameters for countercurrent fermentations

Fermentation Trains	A	B	C
VSLR (g VS/(L liquid•day))	3.49	4.17	4.58
LRT (days)	21	23	25
Total solids fed at each transfer (g)	10.0	12.0	14.0
Air-Lime treated sugarcane trash (g) (pile, second batch)	8.00	9.60	11.2
Chicken Manure (g)	2.00	2.40	2.80
Volatile solids fed to F1 for each transfer (g)	5.88	7.06	8.23
Liquid fed to F4 for each transfer (mL)	100	100	100
F1 retained weight (wet g)	290	288	286
F2-F4 retained weight (wet g)	300	300	300
Temperature (℃)	55.0	55.0	55.0
Frequency of transfer	Every 2 days		
Total iodoform addition rate (μL/day)	100	100	100

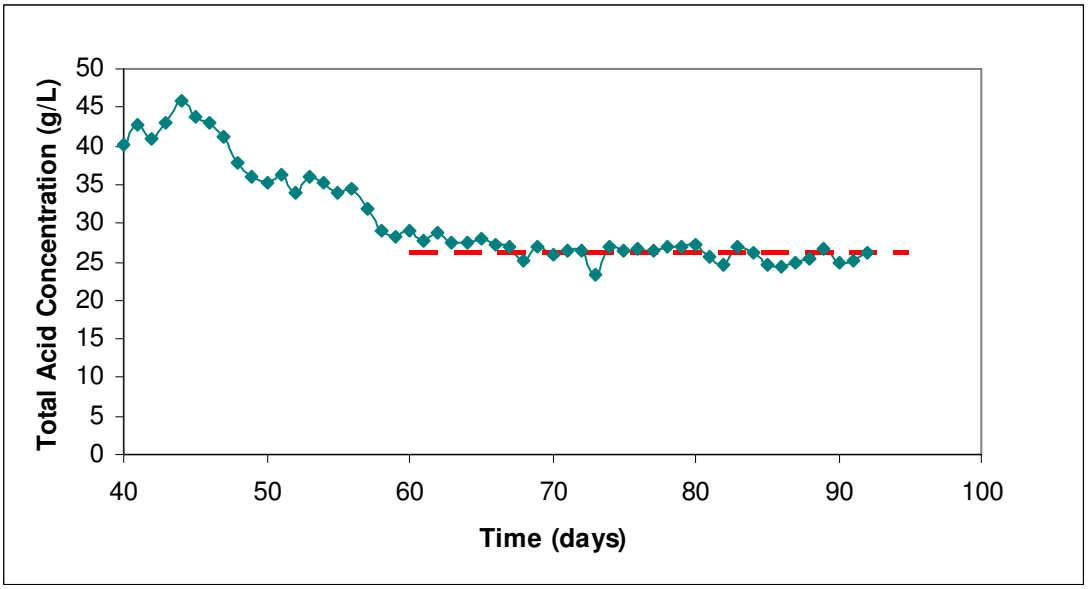


Figure 4-1. Total carboxylic acid concentration in F1 for Train A (VSLR = 3.49 g VS/(L·d) and dash line indicates steady-state value, 26.3 g/L).

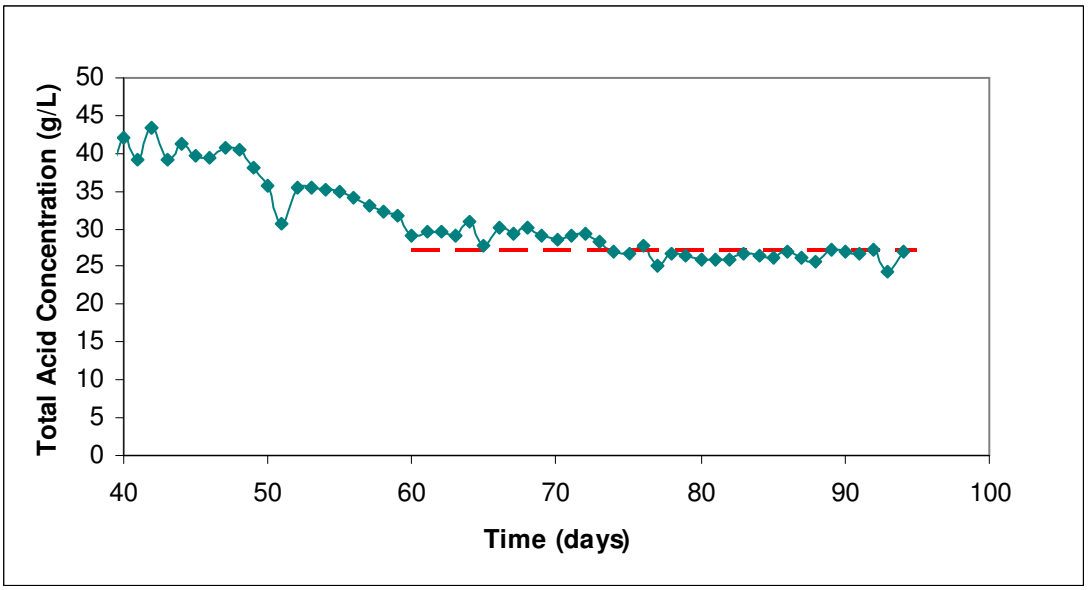


Figure 4-2. Total carboxylic acid concentration in F1 for Train B (VSLR = 4.17 g VS/(L·d) and dash line indicates steady-state value, 27.4 g/L).

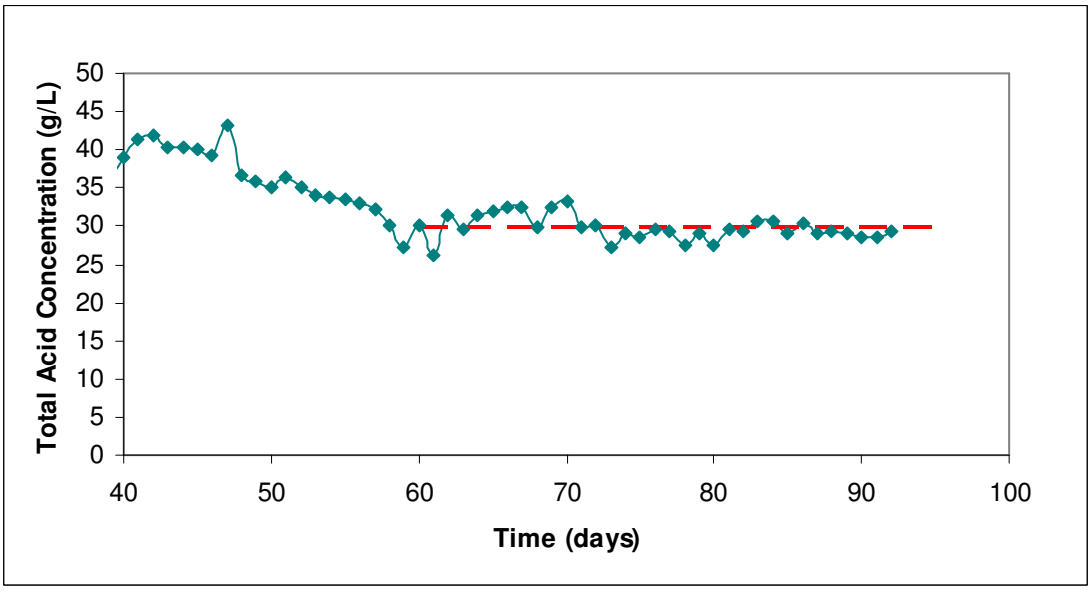


Figure 4-3. Total carboxylic acid concentration in F1 for Train C (VSLR = 4.58 g VS/(L·d) and dash line indicates steady-state value, 29.9 g/L).

Results of the countercurrent fermentations for the three trains are shown in Table 4-2. The highest carboxylic acid productivity of 1.40 g/(L·d) was obtained in Train C (LRT = 25 days and VSLR = 4.58 g VS/(L·d)) at an acid concentration of 29.9 g/L. Fermentation Train A had the highest conversion (0.64 g VS digested/g VS fed), the highest selectivity (0.65 g Aceq/g VS digested), and the highest yield (0.36 g total acid/g VS fed). High yield and conversions obtained in fermentation Train A is due to the low VSLR (3.49 g VS/(L·d)), which uses the fed biomass more completely. The average acetate content in all three trains is close to 80%, which is a characteristic of fermentations buffered by ammonium bicarbonate. Acid production data for the countercurrent fermentation and mass balance calculation are provided in Appendix J.

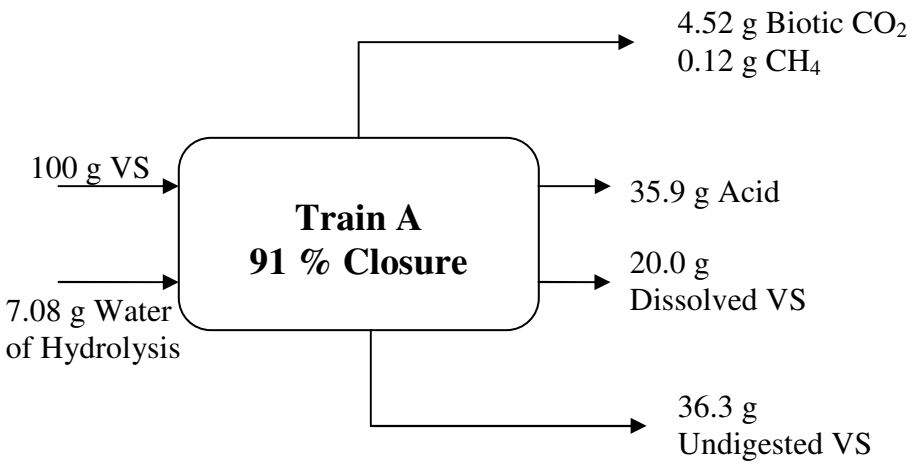
Table 4-2. Results of countercurrent fermentations

Fermentation Trains	A	B	C
Average pH in all fermentors	6.78 ± 0.08	6.80 ± 0.07	6.80 ± 0.07
Total carboxylic acid concentration (g/L)	26.3 ± 1.19	27.4 ± 1.56	29.9 ± 1.53
Acetic acid (wt%)	76.9 ± 3.82	74.6 ± 6.30	74.4 ± 5.28
Propionic acid (wt%)	5.05 ± 0.31	5.59 ± 0.45	5.69 ± 0.54
Butyric acid (wt%)	17.5 ± 2.13	19.3 ± 3.24	19.3 ± 1.67
Valeric acid (wt%)	0.28 ± 0.14	0.23 ± 0.15	0.27 ± 0.14
Caproic acid (wt%)	0.28 ± 0.13	0.27 ± 0.49	0.25 ± 0.13
Heptanoic acid (wt%)	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
Conversion (g VS digested/g VS fed)	0.64	0.59	0.55
Yield (g total acids/g VS fed)	0.359	0.311	0.305
Selectivity (g Aceq/g VS digested)	0.646	0.603	0.602
Total carboxylic acid productivity (g total acid/(L liq·day))	1.26	1.30	1.40
Biotic CO_2 productivity (g CO_2/(L liq·day))	0.158	0.182	0.156
Methane productivity (g CH_4/(L liq.day))	0.004	0.005	0.005
Mass balance closure (g VS out/g VS in)	0.909	0.861	0.884

Note: All errors are ± 1 standard deviation

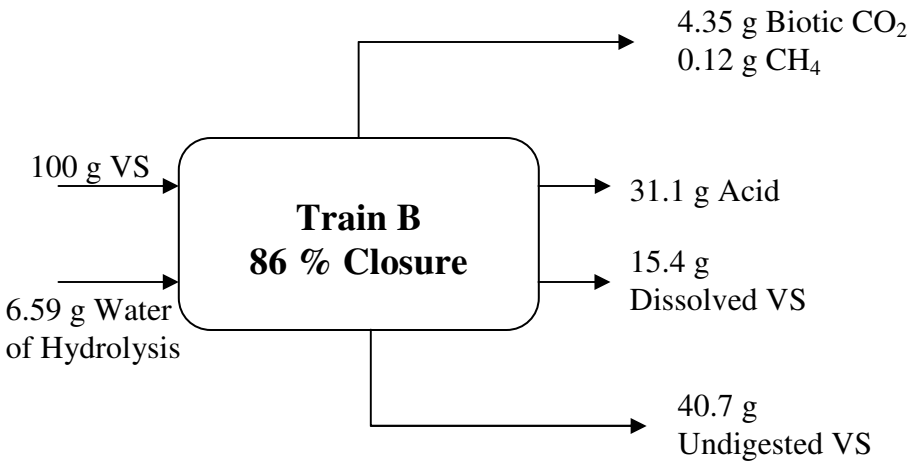
4.3 Mass Balance Closures

Mass balance closure (Equation 2-10) is the ratio of mass exiting the system to mass entering the system. Figure 4-4 shows the mass balance closure for all three trains. Because all mass is being accounted for, the system should theoretically have a 100% closure. However, this is not the case for all the three trains. This error could be attributed to errors in measurements and other discrepancies in the transfer procedure.

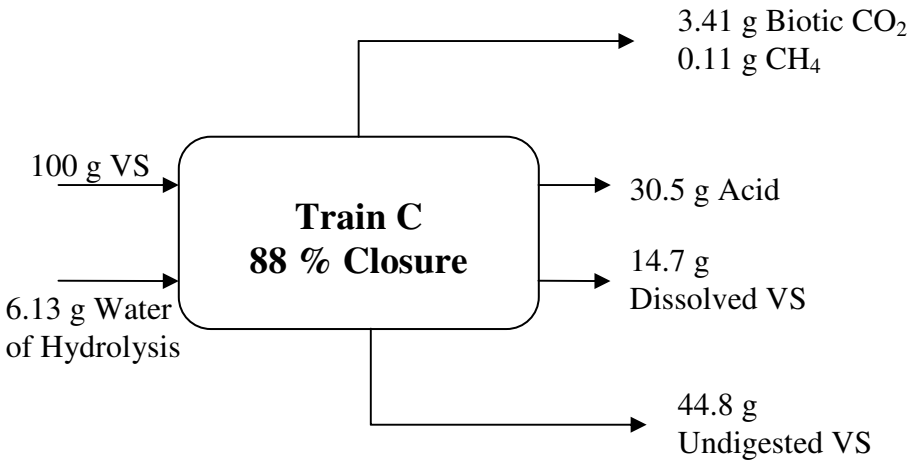


(a) Fermentation Train A mass balance.

Figure 4-4. Mass balance closures for countercurrent fermentations.



(b) Fermentation Train B mass balance.



(c) Fermentation Train C mass balance.

Figure 4-4. (Continued).

4.4 Model Development

CPDM modeling was done to determine the optimum operating conditions for sugarcane trash and chicken manure fermentations. To apply the CPDM method, batch experiments were established at various initial substrate concentrations. The substrate concentrations used were 20, 40, 70, 100, and 100^{+} g dry substrate/L of liquid. The 100 and 100^{+} fermentors had the same initial substrate concentration, but the 100^{+} contained a medium with a mixture of carboxylate salts in a concentration of approximately 20 g of carboxylic acid/L of liquid. The mass composition of salts dissolved in the medium was 80% ammonium acetate, 15% ammonium butyrate, and 5% ammonium propionate. As ammonium butyrate and ammonium propionate were not readily available, these salts were made by reacting their respective acids with ammonium hydroxide solution in stoichiometric amounts. The ratio of carboxylate salts was determined from countercurrent fermentations with sugarcane trash. The liquid medium for these batch experiments was 20% adapted inocula from countercurrent fermentations with sugarcane trash and 80% deoxygenated water. Using adapted inocula is advantageous as the microorganisms are already adapted to the environment and there will be no lag phase associated with acid production. The initial carboxylic acid concentration is associated with the acids already present in the adapted inocula. These batch experiments were run for 10–20 days to determine the rate parameters required in the CPDM model. Liquid samples were collected everyday and 60 μL of iodoform was added every other day. pH adjustment to 7.00–7.25 was done every 2 days and the gas was analyzed periodically for methane production.

The carboxylic acid concentration detected by the Agilent gas chromatograph was converted to acetic acid equivalent concentration (Aceq). Aceq represents the amount of acetic acid that could have been produced in the fermentation if all the carboxylic acids were acetic acid (Datta, 1981). The Aceq unit is based on the reducing power of the acids produced during the fermentation as presented in the following reducing-power-balanced disproportionation reactions (Loescher, 1996). Describing the carboxylic acid concentration as Aceq allows the CPDM method to account for the various carboxylic acids produced as one single parameter. Equations 4-1 to 4-5 show the appropriate disproportionation reactions. Equations 4-6 and 4-7 are used to calculate the Aceq concentration.

Propionic acid: $7\ HOAc \rightarrow 4\ HOPr + 2\ CO_2 + 2\ H_2O$ (4-1)

Butyric acid: $5\ HOAc \rightarrow 2\ HOBu + 2\ CO_2 + 2\ H_2O$ (4-2)

Valeric acid: $13\ HOAc \rightarrow 4\ HOVa + 7\ CO_2 + 6\ H_2O$ (4-3)

Caproic acid: $4\ HOAc \rightarrow HOCa + 2\ CO_2 + 2\ H_2O$ (4-4)

Heptanoic acid $19\ HOAc \rightarrow 4\ HOHe + 10\ CO_2 + 10\ H_2O$ (4-5)

$$\alpha\ (mol/L) = acetic\ (mol/L) + 1.75 \times propionic\ (mol/L) + 2.5 \times butyric\ (mol/L) + 3.25 \times valeric\ (mol/L) + 4.0 \times caproic\ (mol/L) + 4.75 \times heptanoic\ (mol\ /\ L) \quad (4\text{-}6)$$

This can be converted into mass basis:

$$Aceq\ (g/L) = 60.05 \times [\alpha(mol/L)] \quad (4\text{-}7)$$

The Aceq concentrations in each batch experiment was fit to Equation 4-8, where *a, b*, and *c* are constants fit by least square regression using Excel solver and *t* is the time

in days. Initial values of these parameters are guessed for the iterations. These parameters are obtained by minimizing the residuals given as Equation 4-9.

$$\text{Aceq} = a + \frac{bt}{1+ct} \tag{4-8}$$

$$\text{Residuals} = \sum_{\text{data}} (\text{Aceq}_{\text{exp}} - \text{Aceq}_{\text{calculated}})^2 \tag{4-9}$$

Equation 4-8 can be differentiated to give the rate of the reaction, Equation 4-10.

$$\text{Rate} = \frac{\text{d(aceq)}}{\text{dt}} = \frac{b}{(1+ct)^2} \tag{4-10}$$

The reaction rate given shown in Equation 4-10 can be converted to a specific reaction rate (Equation 4-11), by dividing it by the initial substrate concentration S_0 (g VS/L) in the respective batch fermentor.

$$\hat{r} = \frac{r}{S_0} \tag{4-11}$$

The specific rate equation is empirical and Equation 4-12 can be determined by least square analysis.

$$\hat{r}_{pred} = \frac{e(1-x)^f}{1+g(\phi \cdot \text{Aceq})^h} \tag{4-12}$$

The parameters e, f, g, and h are empirical constants and ϕ is the ratio of total grams of actual acid to total grams of Aceq. The biomass conversion $x(t)$ is calculated for each fermentor using Equation 4-13.

$$x(t)=\frac{\text{Aceq}(t)\text{-Aceq}(t=0)}{S_0\sigma} \tag{4-13}$$

where σ is the selectivity (g Aceq produced /g VS digested) for each batch fermentor. In the CPDM method, the selectivity σ is assumed as constant and calculated from the selectivity *s* by Equation 4-14. This *s* is the average value of selectivity (g total acid produced /g VS digested).

$$\sigma=\frac{s}{\phi} \tag{4-14}$$

The parameter ϕ was introduced by Ross (1998) to avoid the inhibitory effects of higher acids that would overestimate the specific reaction rate. The $(1\text{-}x)^f$ term in the numerator is the conversion penalty function described by South and Lynd (1994). It shows that as the conversion increases, the reaction rate decreases. Thus, *f* has to be greater than zero and the larger the magnitude of *f*, the more the reaction rate will decrease.

In conclusion, the batch experiments were established to obtain the parameter values *e*, *f*, *g*, and *h* by least square regression. The other required parameters for the *Mathematica* CPDM program are selectivity (σ), holdup (ratio of liquid to volatile solid in wet cake), moisture (ratio of liquid to volatile solids in feed), and VS concentration in fermentors in g/L. Given these parameters, the CPDM program can predict total Aceq concentration and conversion at various VSLR and LRT. Aceq concentration can be converted back to carboxylic acid concentration by multiplying by ϕ. *Matlab* is used to create a "map" to show the dependence of acid concentration and conversion on the

VSLR and LRT. This allows one to determine the optimum operating conditions for countercurrent fermentations. Data from countercurrent experiments is then used to verify the model prediction and determine the accuracy of prediction. The *Mathematica* and *Matlab* coding for CPDM modeling and "map" creation is provided in Appendix H and Appendix I.

4.5 CPDM Prediction

Batch experiments at varying initial concentrations (20, 40, 70, 100, 100^{+} g/L) were established to predict the reaction rates. Liquid samples from the five fermentors were collected daily for 16 days. The carboxylic acid concentration was analyzed using the gas chromatograph and converted to acetic acid equivalents according to Equations 4-6 and 4-7. The acetic acid equivalent concentration was then fit to Equation 4-8 by least squares regression. Figures 4-5 to 4-9 show the experimental acid profile and the model fit. The numerical values of a, b, and c are shown in Table 4-3.

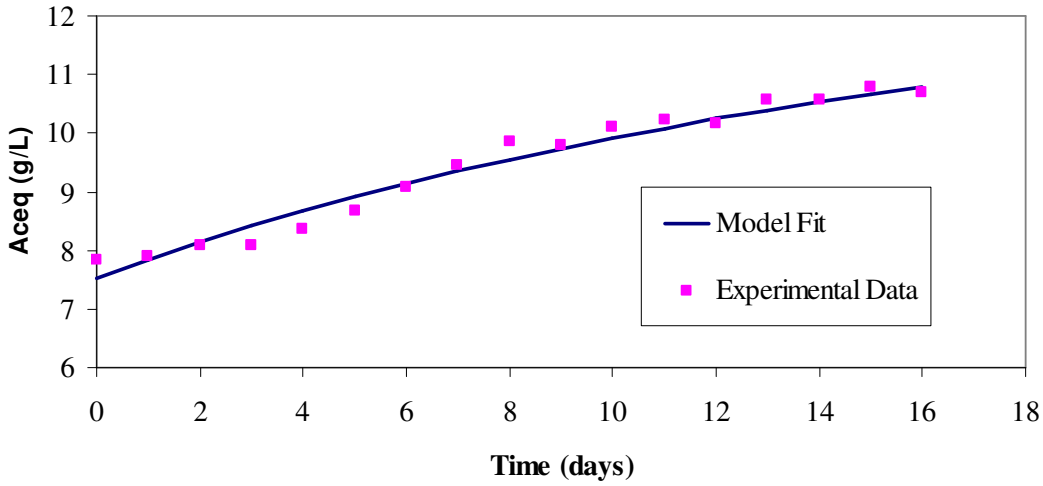


Figure 4-5. Acetic acid equivalent concentration for sugarcane trash/chicken manure batch fermentation (20 g dry substrate/L of liquid).

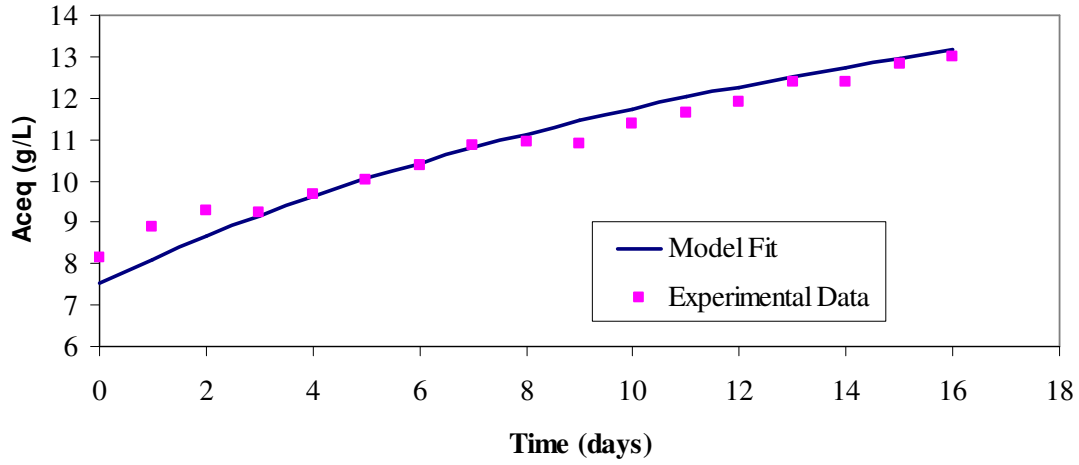


Figure 4-6. Acetic acid equivalent concentration for sugarcane trash/chicken manure batch fermentation (40 g dry substrate/L of liquid).

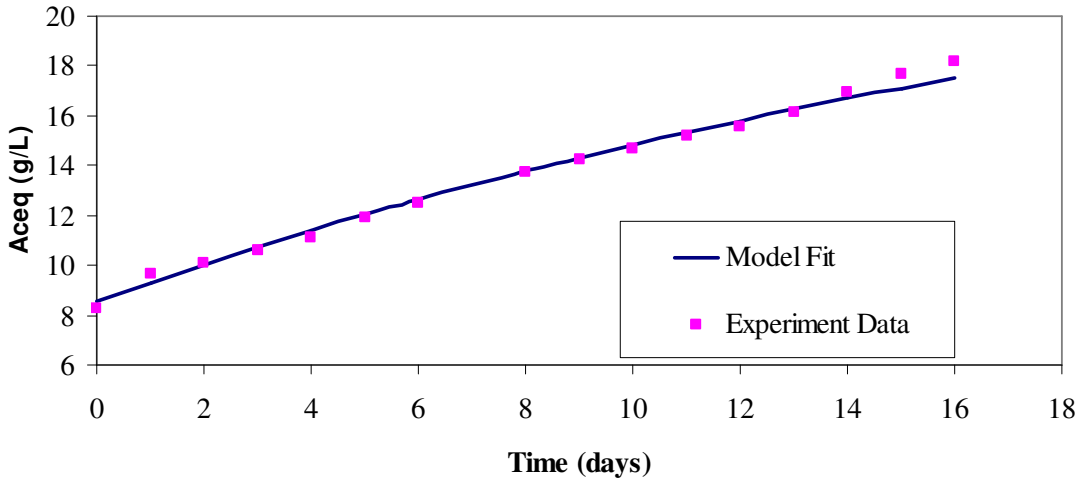


Figure 4-7. Acetic acid equivalent concentration for sugarcane trash/chicken manure batch fermentation (70 g dry substrate/L of liquid).

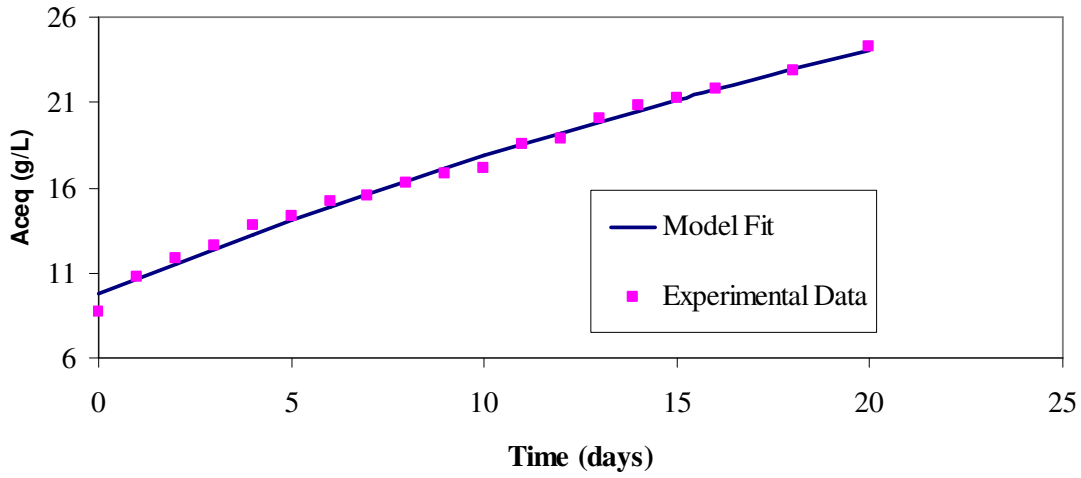


Figure 4-8. Acetic acid equivalent concentration for sugarcane trash/chicken manure batch fermentation (100 g dry substrate/L of liquid).

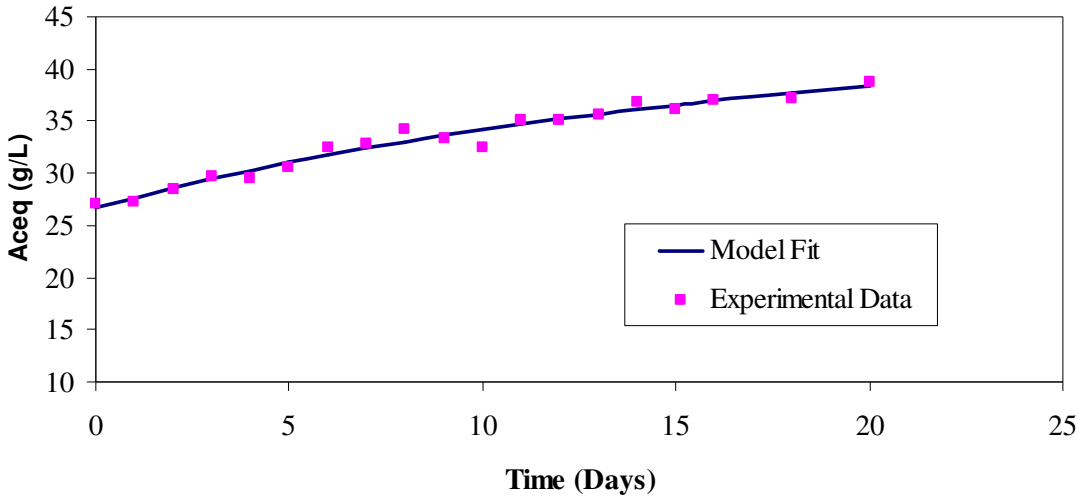


Figure 4-9. Acetic acid equivalent concentration for sugarcane trash/chicken manure batch fermentation (100^{+} g dry substrate/L of liquid).

Table 4-3. The values of *a*, *b*, *c* in CPDM for sugarcane trash and chicken manure fermentation

Initial Substrate Concentration (g/L)	*a* (g/L liquid)	*b* (g /(L liquid·d))	*c* (d^{-1})
20	7.51	0.335	0.04
40	7.52	0.631	0.049
70	8.53	0.787	0.025
100	9.74	0.928	0.015
100^{+}	26.63	1.05	0.039

The values of *e*, *f*, *g*, *h*, and other parameters required for the *Mathematica* program are shown in Table 4-4. The CPDM parameters for the three trains can be calculated using data shown in Appendix K (Tables K-1 to K-3). Results from Fu (2007) on long-term air-lime pretreated bagasse are also presented in Table 4-4. These parameter values are used in the CPDM *Mathematica* program. The program reports

acid concentrations and conversions for user-specified VSLR and LRT points. This enables the user to construct an array within a specific VSLR and LRT range. This array of data is visually represented as a CPDM "map" using *Matlab*. The rate equation for the 80% sugarcane trash/20% chicken manure fermentation with ammonium bicarbonate buffer and marine inoculum is:

$$\hat{r}_{pred} = \frac{0.26(1-x)^{2.66}}{1+3.43(\phi \cdot \text{Aceq})^{0.461}} \tag{4-15}$$

where,

$\hat{r}_{pred}$ = g acetic acid equivalents produced/(g VS·d)

x = conversion (dimensionless)

ϕ = ratio of g total acids to g acetic acid equivalents (dimensionless)

Aceq = g acetic acid equivalent produced/L

Table 4-4. CPDM parameter values for bagasse/chicken manure fermentation and sugarcane trash/chicken manure fermentations

Parameter Constant	Bagasse	Train A Sugarcane Trash	Train B Sugarcane Trash	Train C Sugarcane Trash
Holdup (g liquid/g VS cake)	4.02	4.07	4.41	4.31
Moisture (g liquid/g VS fed)	0.03	0.116	0.116	0.116
Selectivity (g Aceq/g VS digested)	0.72	0.65	0.60	0.60
F1-F4 solids concentration (g VS/L)	159	160	145	150
F1–F4 liquid volume (L)	0.275	0.210	0.211	0.225
Φ (g total acid/g Aceq)	0.9	0.9	0.86	0.86
e (g Aceq/(g VS·d))	0.71	0.26	0.26	0.26
f (dimensionless)	3.19	2.66	2.66	2.66
g (L/g total acid)$^{1/h}$	3.09	3.43	3.43	3.43
h (dimensionless)	0.68	0.461	0.461	0.461

Table 4-5 compares the experimental carboxylic acid concentration in the three trains with the predicted carboxylic acid concentrations. The values shown above in Table 4-4 are used in the *Mathematica* program to predict the acetic acid equivalent concentration, which is converted back to total acid concentration by multiplying by ϕ (ratio of g total acid to g acetic acid equivalents). Because the selectivity varies with the VSLR, individual parameters are used for each train instead of averaging. The average error in the prediction of the carboxylic acid concentration was 4.62%. The highest error in carboxylic acid prediction was 8.77%. The average error in the prediction of conversions was 1.42%. The highest error in the prediction of the conversion was 2.91%.

Table 4-5. Comparison of experimental and predicted acid concentration and conversions for sugarcane trash/chicken manure fermentations

Fermentation Trains	A	B	C	Average ** (%)
Experimental carboxylic acid concentration (g/L)	26.3	27.4	29.9	
Predicted (CPDM) carboxylic acid concentration (g/L)	26.3	26.1	27.2	
Error * (%)	0.34	-4.75	-8.77	4.62
Experimental conversion	0.64	0.59	0.55	
Predicted (CPDM) conversion	0.63	0.57	0.55	
Error * (%)	-1.06	-2.91	-0.27	1.42

Error * (%) = ((Predicted Value-Experimental Value)/Experimental Value)*100

Average**(%) = Average errors are based on absolute values

Figure 4-10 shows the CPDM "map" at the average experimental volatile solids concentration of 150 g VS/L liquid. This CPDM "map" is generated with average values of selectivity, holdup, and liquid volume. The "map" predicts a total acid concentration 37.5 g/L at LRT of 30 days, VSLR of 7 g/(L·d), and conversion of 36%. To obtain 70% conversion and acid concentration of 22 g/L, a VSLR of 3 g/(L·d) and LRT of 19 days is required. At VSLR of 2 g/(L·d) and LRT of 10 days, conversion as high as 86% is predicted.

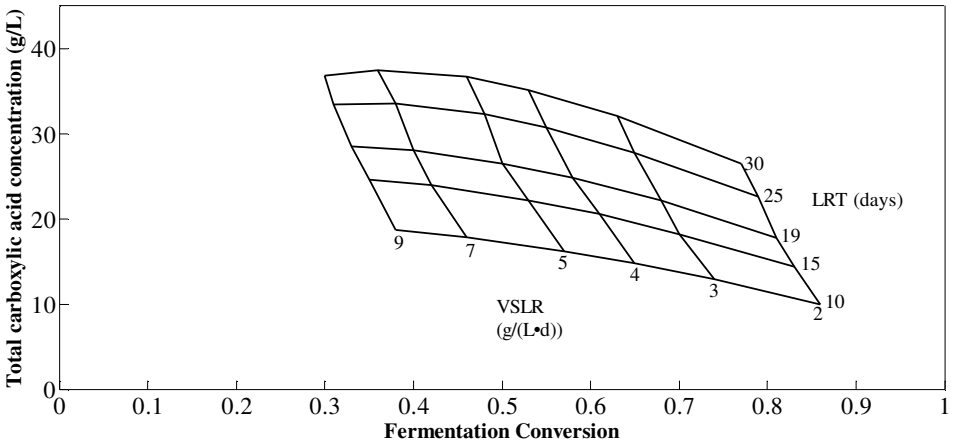


Figure 4-10. CPDM "map" for 80 wt% air-lime pretreated sugarcane trash/20 wt% chicken manure countercurrent fermentation using ammonium bicarbonate buffer at 150 g VS/L of liquid.

High substrate concentrations could be achieved if the process operated on a large scale (Holtzapple, 1999). Typically in the laboratory, a VS concentration of 120–160 g VS/L of liquid is obtained. On an industrial scale, it would be possible to achieve 300 g VS/L of liquid. This would also result in higher carboxylic acid concentrations. Industrial-scale fermentation at 300 g VS/L of liquid was simulated using the CPDM method for sugarcane trash and bagasse fermentations.

Figure 4-11 shows the CPDM "map" for air-lime pretreated sugarcane trash fermentation on an industrial scale (300 g VS/L of liquid). The "map" predicts a total acid concentration of 55.6 g/L at LRT of 30 days, VSLR of 7 g/(L·d), and conversion of 51%. The actual pretreatment yield for long-term air-lime pretreatment is about 70% (i.e., 30% of the solids were solubilized). From Equation 3-3, a fermentation conversion of 70% is required to achieve a target value of 80% overall conversion of volatile solids.

Fermentation conversion of 70% and high acid concentration of 47.5 g/L are predicted at a VSLR of 4 g/(L·d) and LRT of 30 days.

Figure 4-12 shows the CPDM "map" for air-lime pretreated bagasse on an industrial scale (300 g VS/L of liquid). The "map" predicts a total acid concentration of 62.4 g/L at LRT of 30 days, VSLR of 7 g/(L·d), and conversion of 51%. Fermentation conversion of 70%, and high acid concentration of 52 g/L are predicted at a VSLR of 4 g/(L·d) and LRT of 30 days.

Figure 4-13 compares the fermentation behavior of both substrates. From the analysis of Figure 4-13, bagasse tends to have slightly higher acid concentrations. The air-lime pretreated sugarcane trash was washed after neutralization. This could have removed some of the volatiles and caused the inferior performance of the sugarcane trash/chicken manure fermentation. The conversions in both fermentations are similar at low VSLR. Increasing the VSLR, results in higher conversions in the bagasse fermentation.

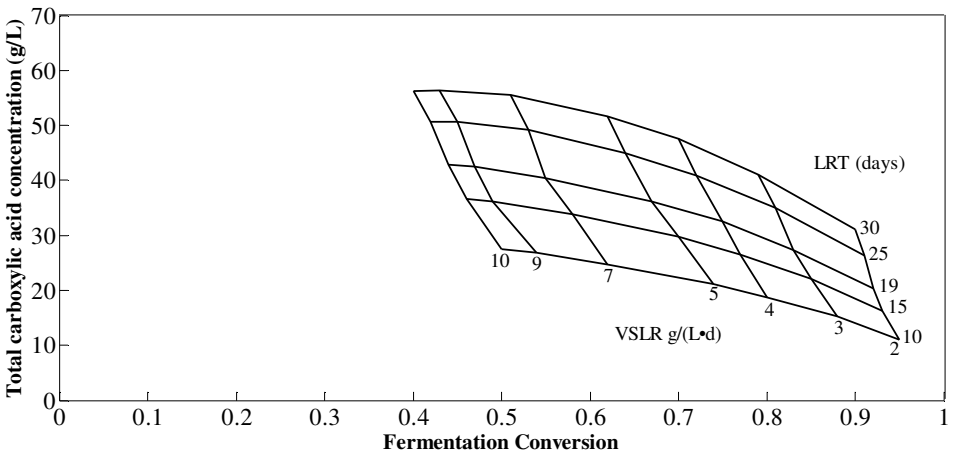


Figure 4-11. CPDM "map" for 80 wt% air-lime pretreated sugarcane trash/20 wt% chicken manure countercurrent fermentation using ammonium bicarbonate buffer at 300 g VS/L of liquid.

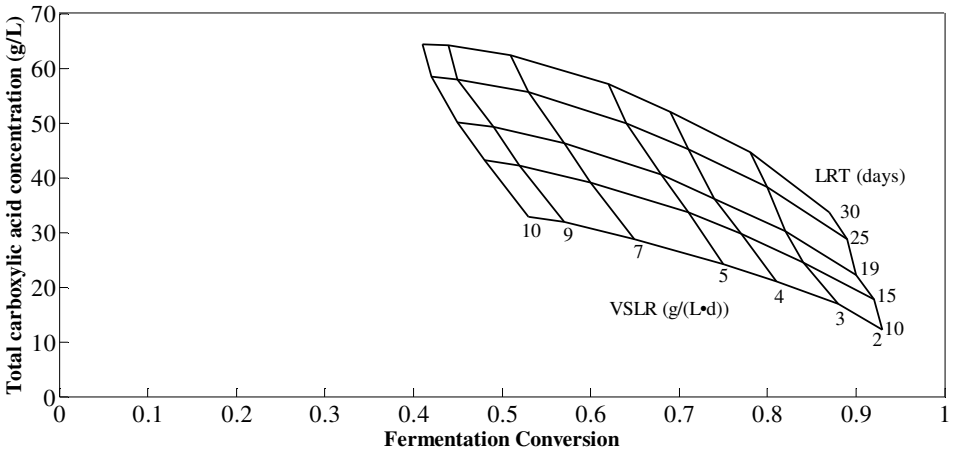


Figure 4-12. CPDM "map" for 80 wt% air-lime pretreated bagasse/20 wt% chicken manure countercurrent fermentation using ammonium bicarbonate buffer at 300 g VS/L of liquid.

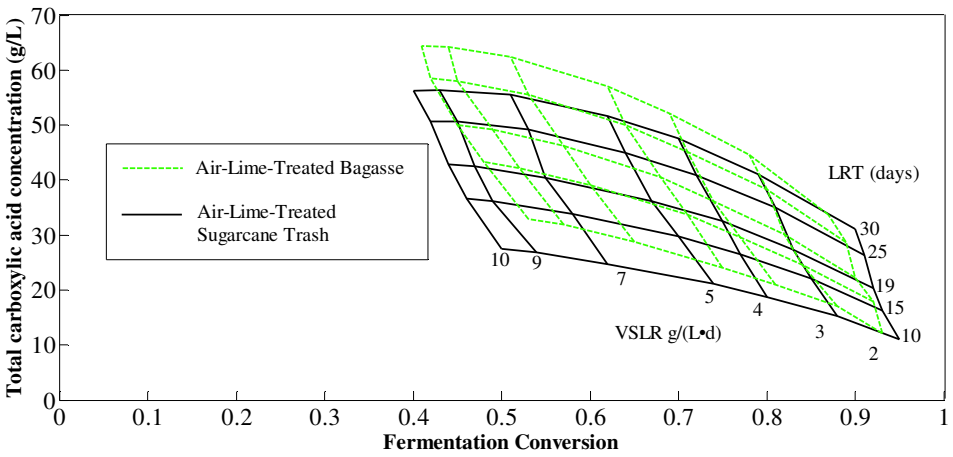


Figure 4-13. CPDM "map" for both air-lime pretreated 80 wt% bagasse and sugarcane trash/20 wt% chicken manure countercurrent fermentation using ammonium bicarbonate buffer at 300 g VS/L of liquid.

4.6 Conclusions

The highest acid productivity for the 80 wt% air-lime-treated sugarcane trash/20 wt% chicken manure fermentation of 1.40 g total acid/(L liquid·day) was obtained in Train C with VSLR of 4.58 g VS/(L·d) and LRT of 25 days. CPDM predicted acid concentrations and conversions with an average error of 4.62% and 1.42% respectively. Countercurrent fermentations require many months to reach steady state. CPDM is a valid and reliable way to determine optimum conditions for operating countercurrent fermentations. This can save a lot of time as only simple batch experiments at varying initial solids concentration are required to determine the parameters required for the CPDM program. The CPDM "map" for an industrial-scale (300 g VS/L) air-lime pile pretreated sugarcane trash fermentation predicts a total acid concentration of 55.6 g/L at LRT of 30 days, VSLR of 7 g/(L·d), and conversion of 51%. Fermentation conversion of

70% and high acid concentration of 47.5 g/L are predicted at a VSLR of 4 g/(L·d) and LRT of 30 days. Comparison of air-lime pretreated sugarcane trash performance with that of air-lime pretreated bagasse in countercurrent fermentations shows sugarcane trash to be a slightly inferior substrate choice. The pretreatment method used greatly affects the acid productivity of biomass substrates. The next chapter will investigate some other forms of chemical and physical pretreatments. The performance of these pretreatments will be evaluated using the CPDM method described in this chapter. CPDM maps generated will be compared to determine the best pretreatment method for the sugarcane trash substrate.

CHAPTER V

PRETREATMENT EVALUATION USING CPDM

5.1 Introduction

Enzymatic simultaneous saccharification and fermentation (SSF) uses a complex mixture of cellulase enzymes to produce soluble monosacharides from the treated biomass. The sugars are then simultaneously fermented to ethanol to reduce the effect of product (sugar) inhibition and increase the enzymatic hydrolysis rates.

There are several biomass features that can affect enzymatic hydrolysis. These are namely lignin content, cellulose crystallinity, presence of acetyl groups, surface area/pore volume of cellulose fiber, and particle size (Sewalt et al., 1997; Converse et al., 1990; Wong et al., 1988). Biomass pretreatment is necessary to alter some of these chemical and physical properties to make the biomass more digestible. The effectiveness of a particular pretreatment is usually assessed by enzymatic hydrolysis. Both Kim (2004) and Sierra-Ramirez (2005) used enzymatic hydrolysis to measure the effectiveness of oxidative long-term lime treatment on corn stover and poplar wood, respectively. The enzymatic hydrolysis yield is reported as g glucan/xylan hydrolyzed per 100 g glucan/xylan in the treated biomass.

In this study, instead of using the traditional enzymatic hydrolysis route, the proven CPDM method (Fu, 2007; Agbogbo, 2005; Aiello Mazzarri, 2002; Thanokoses, 2002) was used to compare the effectiveness of several pretreatments. The maps generated using the CPDM method were used to compare and determine the most favorable pretreatment in terms of carboxylic acid production.

5.2 Materials and Methods

Sugarcane trash was used as the biomass substrate in all these experiments. The sugarcane trash was hammer milled at Cater Mattil Hall, Texas A&M University. Chicken manure was used as the nutrient source. Table 5-1 describes the different pretreatments which were performed. These pretreatments are described in more detail in Chapter II (Section 2.2). Table 2-1 lists the compositional properties such as ash content, lignin content, moisture content, and volatile solids content.

The CPDM method presented in Chapter II was used to develop the model and obtain the CPDM parameters. Batch experiments at initial solids concentrations of 20, 40, 70, 100, and 100^{+} g/L with 80 wt% pretreated sugarcane trash and 20 wt% dried chicken manure were established and data were collected for 10–20 days. The experimental acetic acid equivalent concentrations were fit to Equation 4-8 to determine the parameters *a*, *b*, and *c*. The specific reaction rate is an empirical equation and is fit by non-linear regression to Equation 4-12. Once the parameters *e*, *f*, *g*, and *h* have been determined, the *Mathematica* CPDM program is used to predict acid concentrations and conversions. These data were collected for various VSLR and LRT. The array of data were represented as the CPDM "map" for each of the pretreatments investigated. The maps were generated with a volatile solids concentration of 300 g VS/L liquid to represent an industrial-scale fermentation system.

Table 5-1. Description of pretreatments performed

Pretreatment method	Description
Long-term wash **(LTW)**	Long-term air-lime pile pretreated sugarcane trash used in the countercurrent experiments. The pretreated sugarcane trash was neutralized with carbon dioxide and thoroughly washed using distilled water. The pretreatment liquid was not harvested.
Long-term no-wash **(LTNW)**	Long-term, submerged, air-lime pretreated sugarcane trash. The pretreated sugarcane trash was harvested together with the pretreatment liquid and neutralized using carbon dioxide. The pretreated biomass was then air dried with no washing.
Short-term wash **(STW)**	Short-term oxidative lime-treated sugarcane trash. The pretreated sugarcane trash was neutralized using an acetic acid mix-stir-centrifuge wash cycle.
Short-term no-wash **(STNW)**	Short-term oxidative lime-treated sugarcane trash. The pretreated sugarcane trash was neutralized using carbon dioxide. There was no washing and the biomass was air dried.
Short-term wash ball-milled **(STW-BM)**	Short-term oxidative lime-treated sugarcane trash. The pretreated sugarcane trash was neutralized using an acetic acid mix-stir-centrifuge wash cycle. The samples were air dried and ball-milled for 3 days.
Short-term no-wash ball-milled **(STNW-BM)**	Short-term oxidative lime-treated sugarcane trash. The pretreated sugarcane trash was neutralized using carbon dioxide. There was no washing and the biomass was air dried. The air dried biomass was ball-milled for 3 days.

5.3 CPDM Prediction

This section presents all the results for the individual pretreatments described in Table 5-1.

5.3.1 CPDM Prediction for LTNW

The results for the long-term air-lime pile pretreated (LTW) sugarcane trash have already been presented in the previous chapter. Figures 5-1 to 5-5 show the experimental acid profile and the model fit for the long-term, submerged, air-lime pretreated (LTNW)

sugarcane trash/chicken manure batch fermentations. Table 5-2 presents the values of the fitted parameters *a, b,* and *c*. The predicted specific reaction rate is shown in Equation 5-1. The CPDM parameters required for the *Mathematica* program are given in Table 5-3.

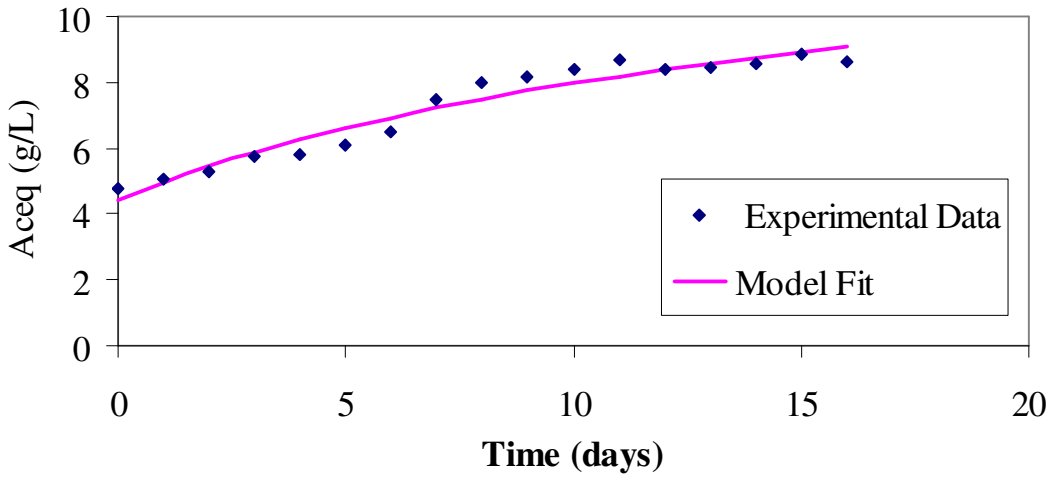


Figure 5-1. Acetic acid equivalent concentration for LTNW batch fermentation (20 g dry substrate/L of liquid).

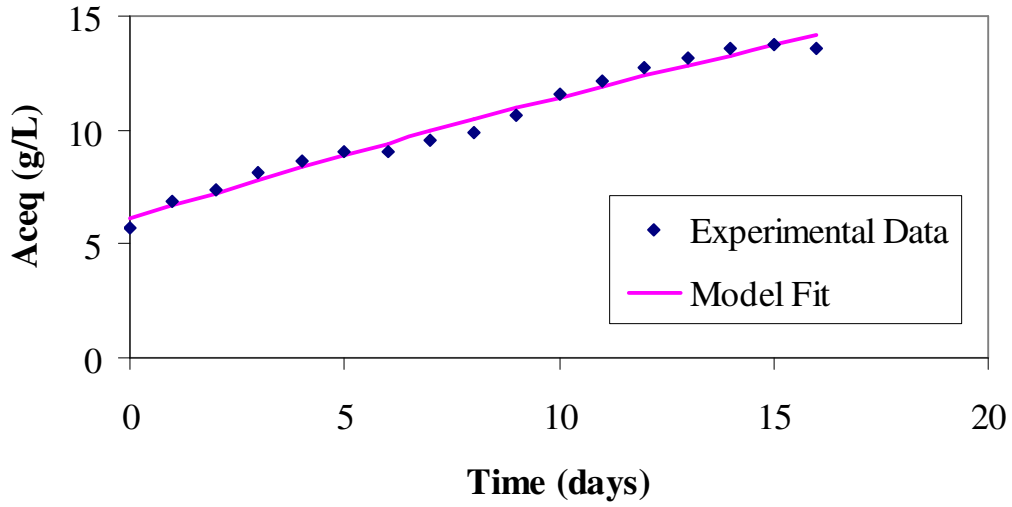


Figure 5-2. Acetic acid equivalent concentration for LTNW batch fermentation (40 g dry substrate/L of liquid).

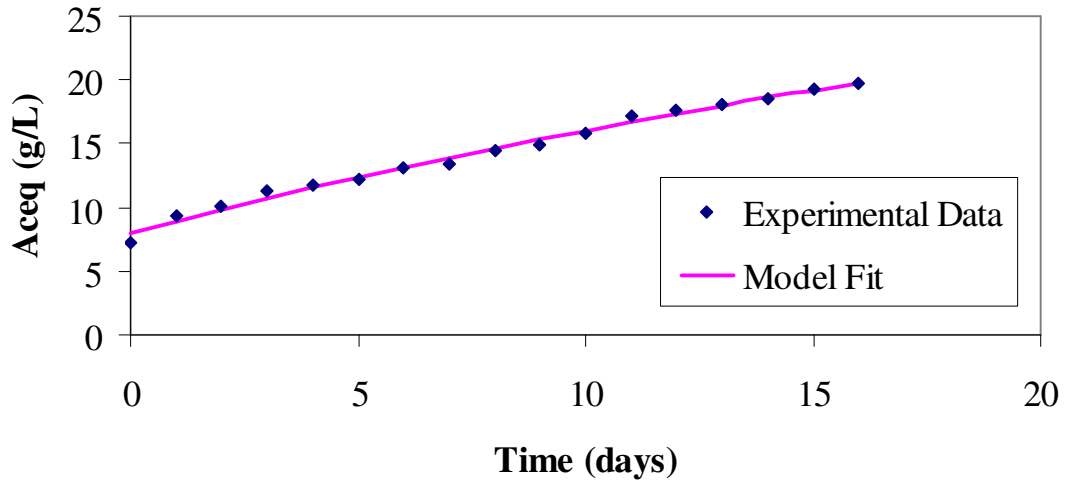


Figure 5-3. Acetic acid equivalent concentration for LTNW batch fermentation (70 g dry substrate/L of liquid).

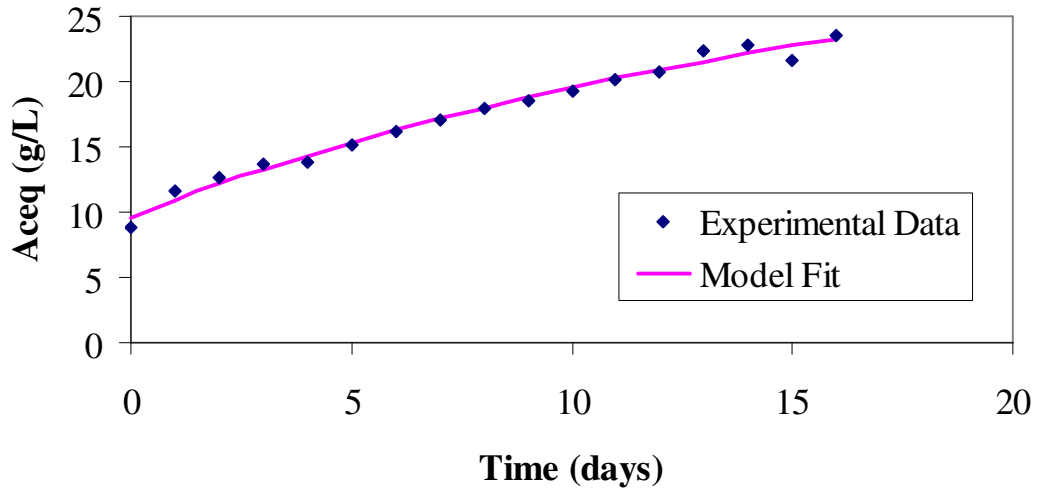


Figure 5-4. Acetic acid equivalent concentration for LTNW batch fermentation (100 g dry substrate/L of liquid).

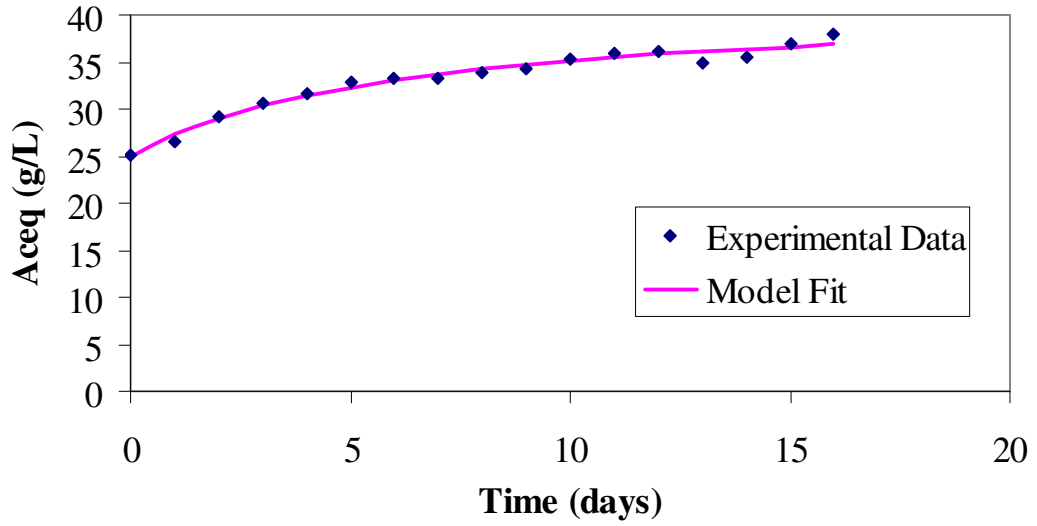


Figure 5-5. Acetic acid equivalent concentration for LTNW batch fermentation (100^{+} g dry substrate/L of liquid).

Table 5-2. The values of *a*, *b*, and *c* for LTNW batch fermentation

Initial Substrate Concentration (g/L)	*a* (g/L liquid)	*b* (g /(L liquid·d))	*c* (d^{-1})
20	4.43	0.561	0.059
40	6.08	0.595	0.011
70	8.00	0.943	0.018
100	9.62	1.346	0.036
100^{+}	24.97	2.648	0.160

The predicted specific rate equation is:

$$\hat{r}_{pred} = \frac{0.765(1-x)^{2.89}}{1+4.22(\phi \cdot \text{Aceq})^{0.67}} \tag{5-1}$$

Table 5-3. Parameter constant values in CPDM for LTNW sugarcane trash/chicken manure fermentation with ammonium bicarbonate

Parameter Constant	Long-Term No-Wash
Holdup (g liquid/g VS cake)	4.27
Moisture (g liquid/g VS fed)	0.116
Selectivity (g Aceq/g VS digested)	0.646
F1–F4 solids concentration (g VS/L)	300
F1–F4 liquid volume (L)	0.21
Φ (g total acid/g Aceq)	0.9
e (g Aceq/(g VS·d))	0.765
f (dimensionless)	2.89
g (L/g total acid)$^{1/h}$	4.22
h (dimensionless)	0.67

Figure 5-6 shows the CPDM "map" for the LTNW sugarcane trash/chicken manure fermentation system at 300 g VS/L liquid. The "map" predicts a total acid concentration of 57.6 g/L at LRT of 30 days, VSLR of 7 g/(L·d), and conversion of 53%.

High conversion of 75% and high acid concentration of 43 g/L are achieved at a VSLR of 4 g/(L·d) and LRT of 25 days.

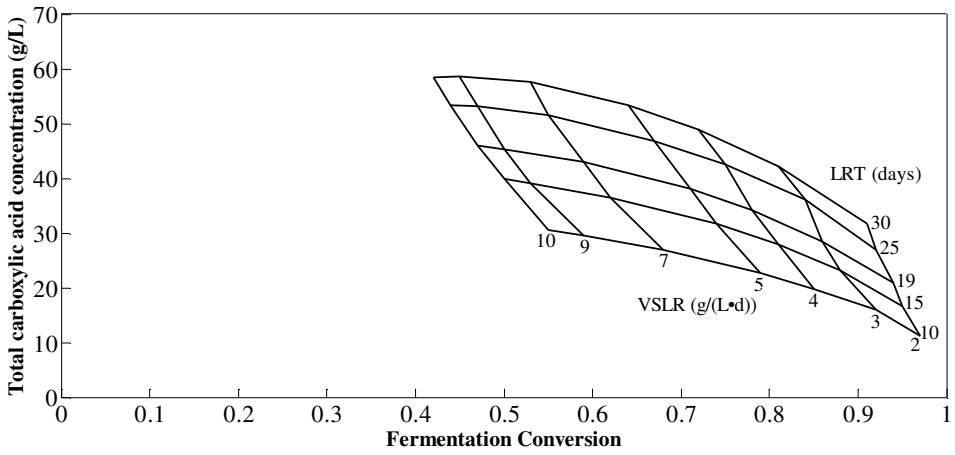


Figure 5-6. CPDM "map" for 80 wt% LTNW sugarcane trash/20 wt% chicken manure countercurrent fermentation using ammonium bicarbonate buffer at 300 g VS/L of liquid.

5.3.2 CPDM Prediction for STW

Figures 5-7 to 5-11 show the experimental acid profile and the model fit for the short-term, acid-washed, oxidative lime pretreated (STW) sugarcane trash/chicken manure batch fermentations. Table 5-4 presents the values of the fitted parameters *a*, *b*, and *c*. The predicted specific reaction rate is shown in Equation 5-2. The CPDM parameters required for the *Mathematica* program are given in Table 5-5.

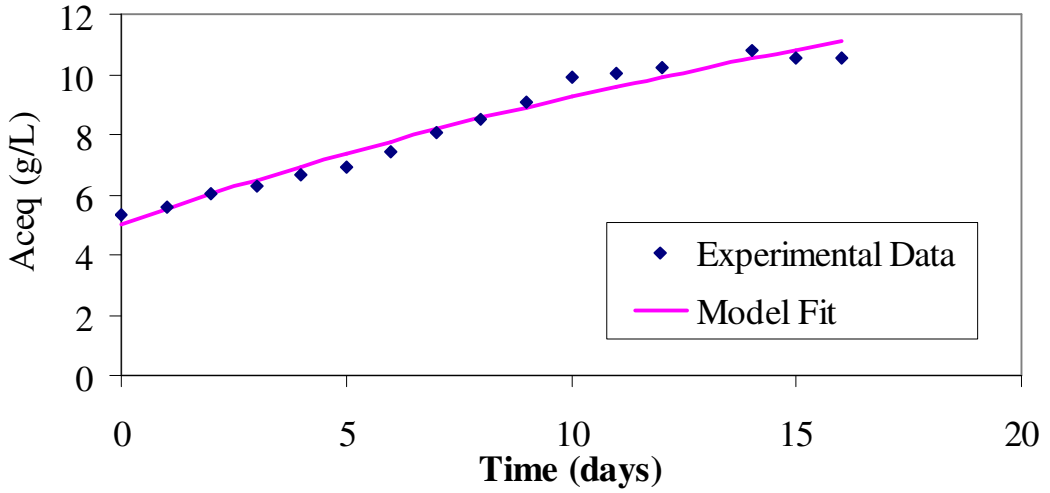


Figure 5-7. Acetic acid equivalent concentration for STW batch fermentation (20 g dry substrate/L of liquid).

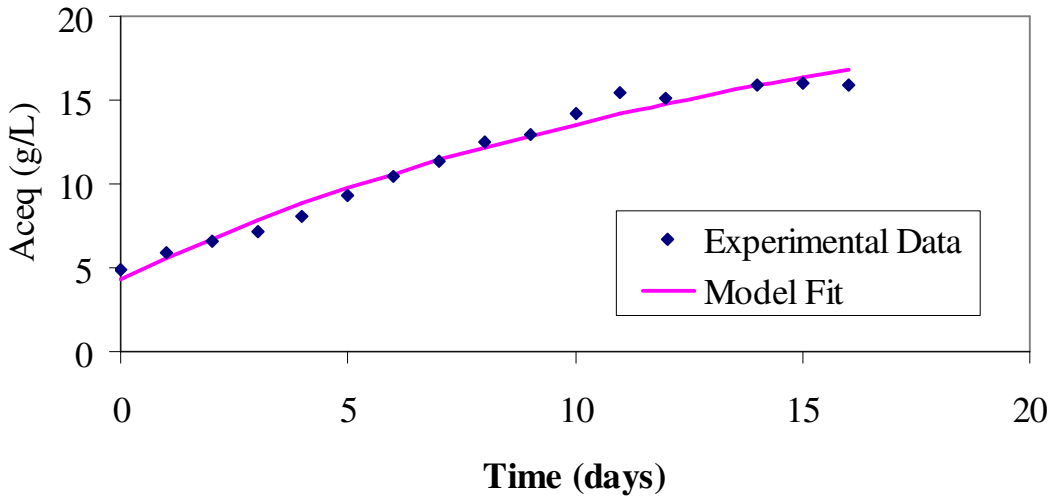


Figure 5-8. Acetic acid equivalent concentration for STW batch fermentation (40 g dry substrate/L of liquid).

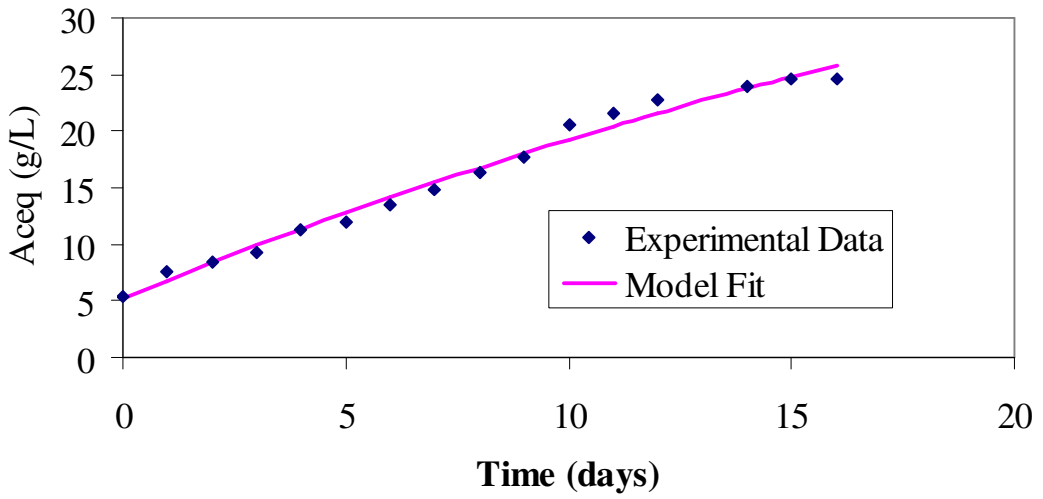


Figure 5-9. Acetic acid equivalent concentration for STW batch fermentation (70 g dry substrate/L of liquid).

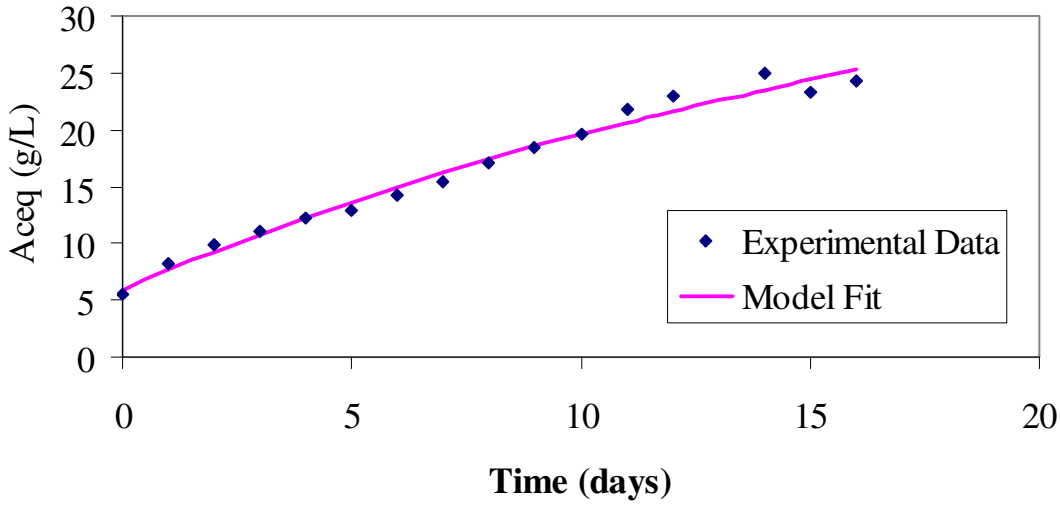


Figure 5-10. Acetic acid equivalent concentration for STW batch fermentation (100 g dry substrate/L of liquid).

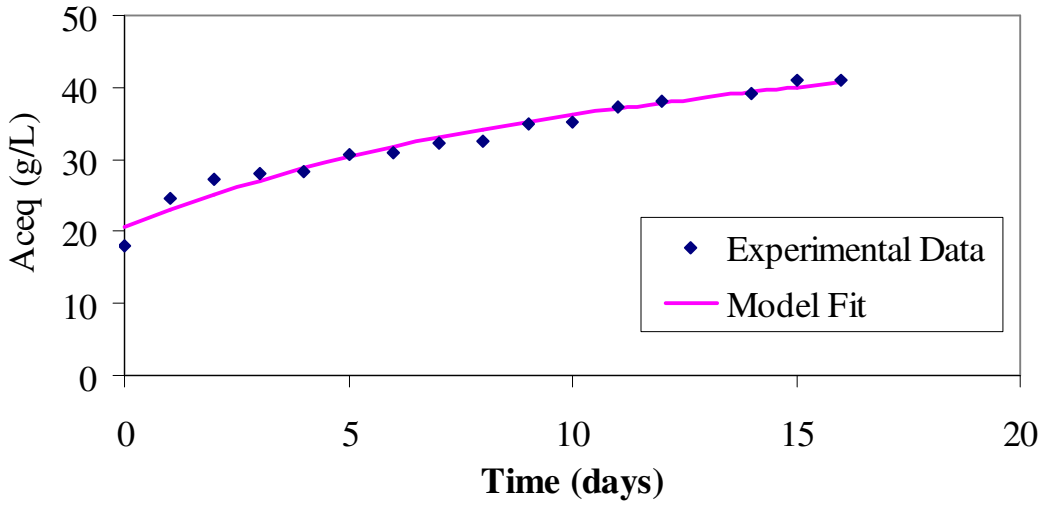


Figure 5-11. Acetic acid equivalent concentration for STW batch fermentation (100^{+} g dry substrate/L of liquid).

Table 5-4. The values of *a*, *b*, and *c* for STW batch fermentation

Initial Substrate Concentration (g/L)	*a* (g/L liquid)	*b* (g /(L liquid·d))	*c* (d^{-1})
20	5.00	0.531	0.025
40	4.31	1.316	0.043
70	5.21	1.632	0.017
100	5.94	1.762	0.029
100^{+}	20.53	2.633	0.069

The predicted specific rate equation is:

$$\hat{r}_{pred} = \frac{0.564(1-x)^{3.12}}{1+4.72(\phi \cdot \mathrm{Aceq})^{0.55}} \qquad (5\text{-}2)$$

Table 5-5. Parameter constant values in CPDM for STW sugarcane trash/chicken manure fermentation with ammonium bicarbonate

Parameter Constant	Short-Term Wash
Holdup (g liquid/g VS cake)	4.27
Moisture (g liquid/g VS fed)	0.113
Selectivity (g Aceq/g VS digested)	0.646
F1–F4 solids concentration (g VS/L)	300
F1–F4 liquid volume (L)	0.21
Φ (g total acid/g Aceq)	0.9
e (g Aceq/(g VS·d))	0.564
f (dimensionless)	3.116
g (L/g total acid)$^{1/h}$	4.72
h (dimensionless)	0.55

Figure 5-12 shows the CPDM "map" for the STW sugarcane trash/chicken manure fermentation system at 300 g VS/L liquid. The "map" predicts a total acid concentration of 55.8 g/L at LRT of 30 days, VSLR of 7 g/(L·d), and conversion of 51%. The pretreatment yield of solids for bagasse under similar conditions was determined by Sierra-Ramirez (PhD student) to be approximately 90% for the short-term pretreatments. For an overall conversion of 80%, a fermentation conversion of approximately 77% will be required. High conversion of 70% and high acid concentration of 40 g/L are achieved at a VSLR of 4 g/(L·d) and LRT of 25 days.

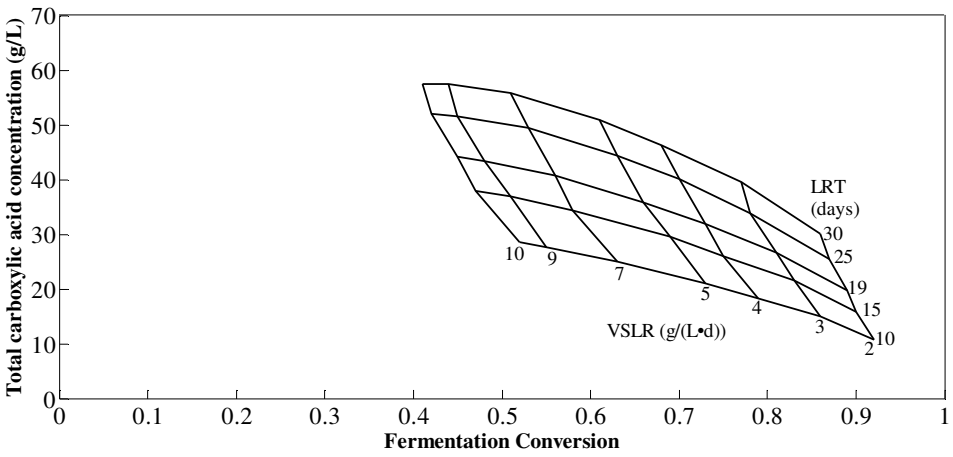


Figure 5-12. CPDM "map" for 80 wt% STW sugarcane trash/20 wt% chicken manure countercurrent fermentation using ammonium bicarbonate buffer at 300 g VS/L of liquid.

5.3.3 CPDM Prediction for STNW

Figures 5-13 to 5-17 show the experimental acid profile and the model fit for the short-term, no-wash, oxidative lime pretreated (STNW) sugarcane trash/chicken manure batch fermentations. Table 5-6 presents the values of the fitted parameters *a*, *b*, and *c*. The predicted specific reaction rate is shown in Equation 5-3. The CPDM parameters required for the *Mathematica* program are given in Table 5-7.

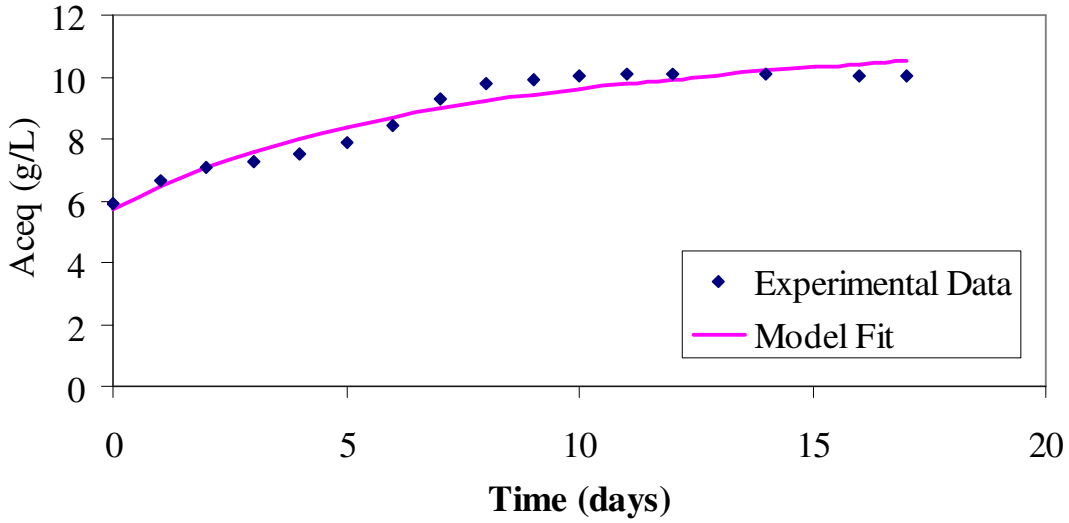


Figure 5-13. Acetic acid equivalent concentration for STNW batch fermentation (20 g dry substrate/L of liquid).

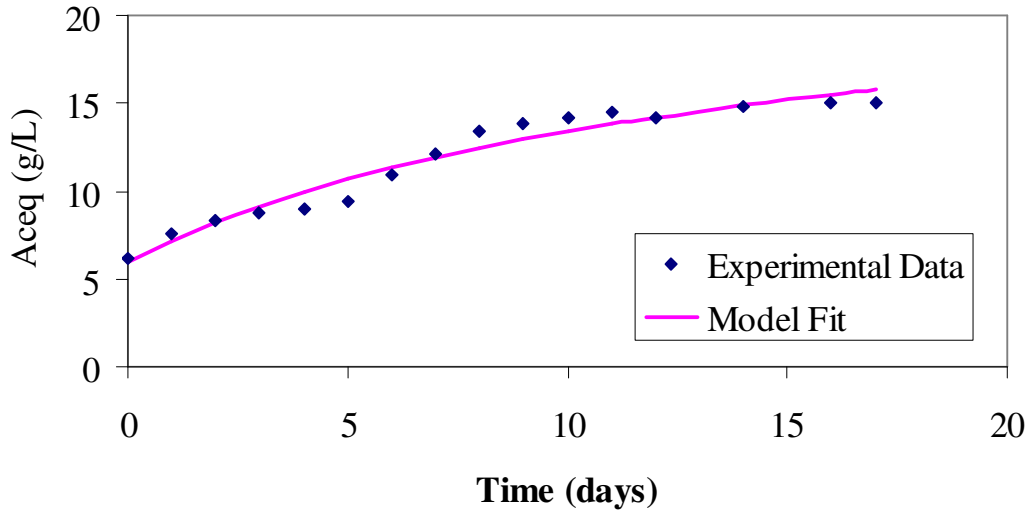


Figure 5-14. Acetic acid equivalent concentration for STNW batch fermentation (40 g dry substrate/L of liquid).

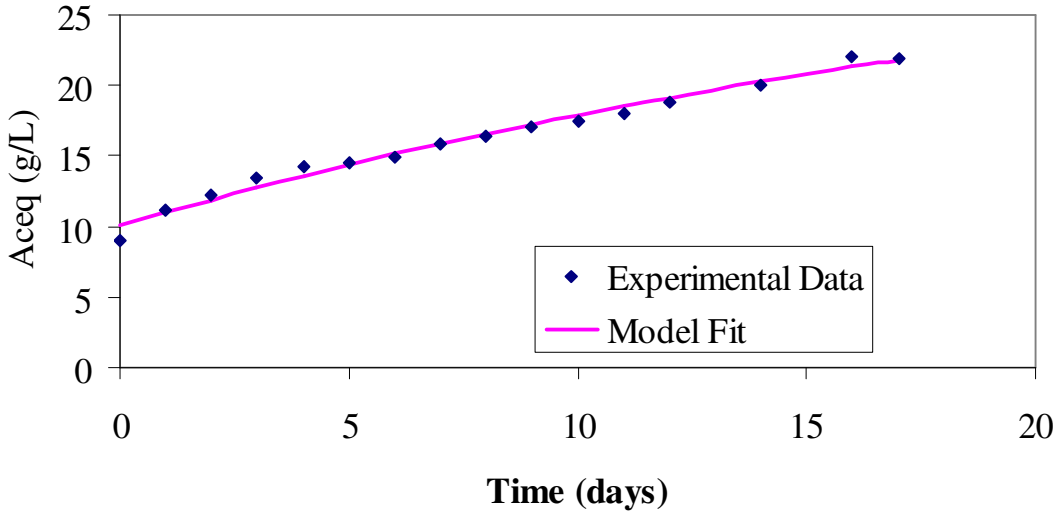


Figure 5-15. Acetic acid equivalent concentration for STNW batch fermentation (70 g dry substrate/L of liquid).

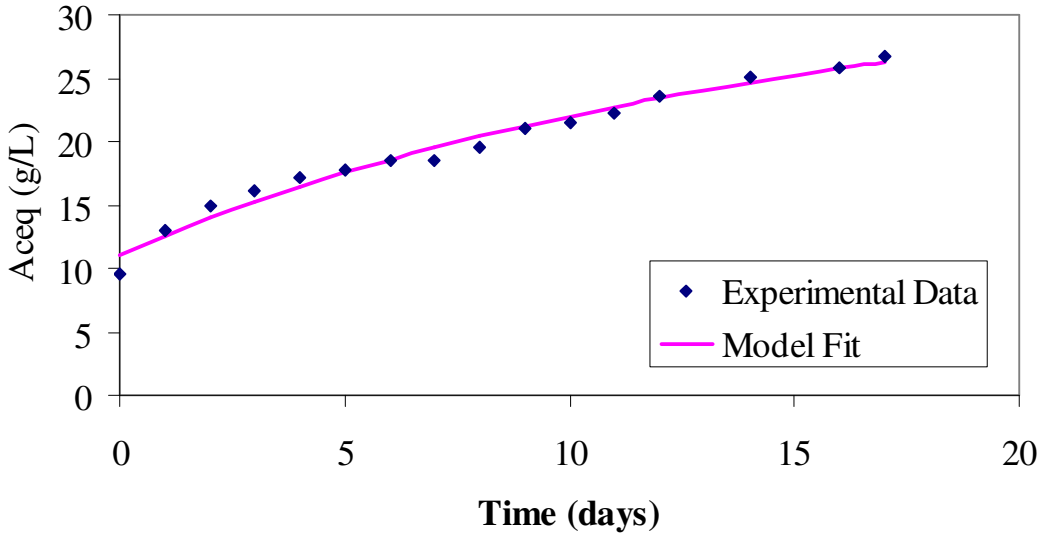


Figure 5-16. Acetic acid equivalent concentration for STNW batch fermentation (100 g dry substrate/L of liquid).

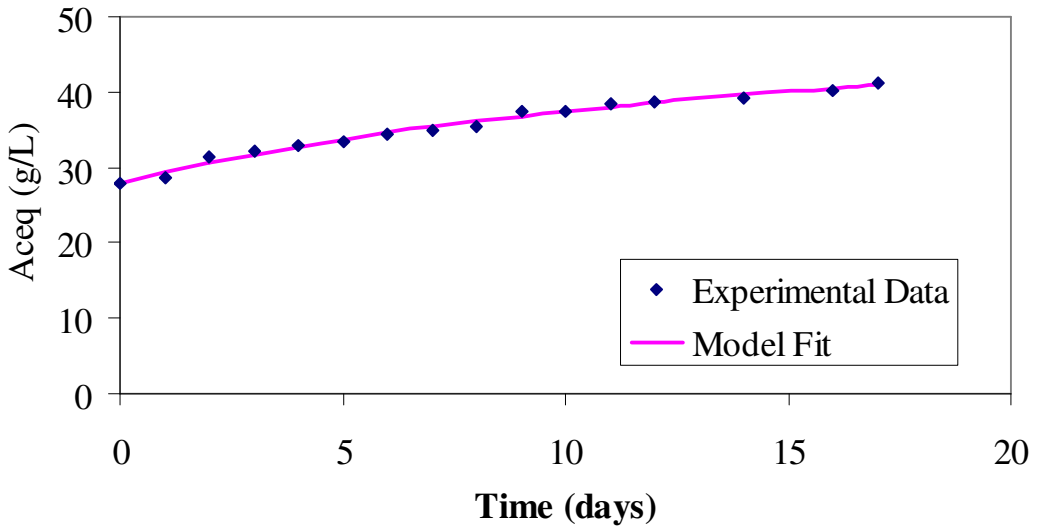


Figure 5-17. Acetic acid equivalent concentration for STNW batch fermentation (100^{+} g dry substrate/L of liquid).

Table 5-6. The values of *a*, *b*, and *c* for STNW batch fermentation

Initial Substrate Concentration (g/L)	*a* (g/L liquid)	*b* (g /(L liquid·d))	*c* (d^{-1})
20	5.74	0.834	0.116
40	5.92	1.299	0.073
70	10.04	0.970	0.024
100	11.05	1.603	0.047
100^{+}	27.98	1.456	0.054

The predicted specific rate equation is:

$$\hat{r}_{pred} = \frac{0.854(1-x)^{3.25}}{1+5.627(\phi \cdot \text{Aceq})^{0.58}} \tag{5-3}$$

Table 5-7. Parameter constant values in CPDM for STNW sugarcane trash/chicken manure fermentation with ammonium bicarbonate

Parameter Constant	Short-Term No-Wash
Holdup (g liquid/g VS cake)	4.27
Moisture (g liquid/g VS fed)	0.18
Selectivity (g Aceq/g VS digested)	0.646
F1–F4 solids concentration (g VS/L)	300
F1–F4 liquid volume (L)	0.21
Φ (g total acid/g Aceq)	0.9
e (g Aceq/(g VS·d))	0.854
f (dimensionless)	3.25
g (L/g total acid)$^{1/h}$	5.627
h (dimensionless)	0.58

Figure 5-18 shows the CPDM "map" for the STNW sugarcane trash/chicken manure fermentation system at 300 g VS/L liquid. The "map" predicts a total acid concentrations of 58 g/L at LRT of 30 days, VSLR of 7 g/(L·d), and conversion of 53%. High conversion of 80% and high acid concentration of 40 g/L are achieved at a VSLR of 3 g/(L·d) and LRT of 30 days.

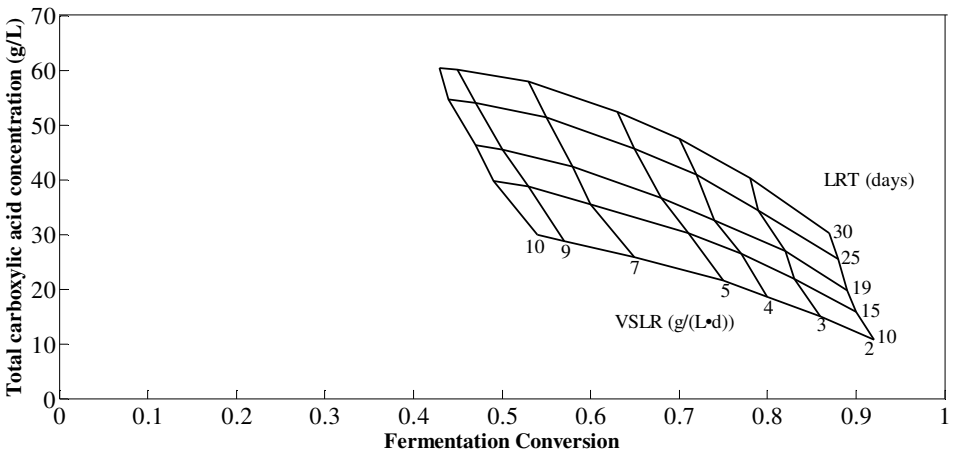


Figure 5-18. CPDM "map" for 80 wt% STNW sugarcane trash/20 wt% chicken manure countercurrent fermentation using ammonium bicarbonate buffer at 300 g VS/L of liquid.

5.3.4 CPDM Prediction for STW-BM

Figures 5-19 to 5-23 show the experimental acid profile and the model fit for the short-term, acid-washed, oxidative lime pretreated, and ball-milled (STW-BM) sugarcane trash/chicken manure batch fermentations. Table 5-8 presents the values of the fitted parameters *a*, *b*, and *c*. The predicted specific reaction rate is shown in Equation 5-4. The CPDM parameters required for the *Mathematica* program are given in Table 5-9.

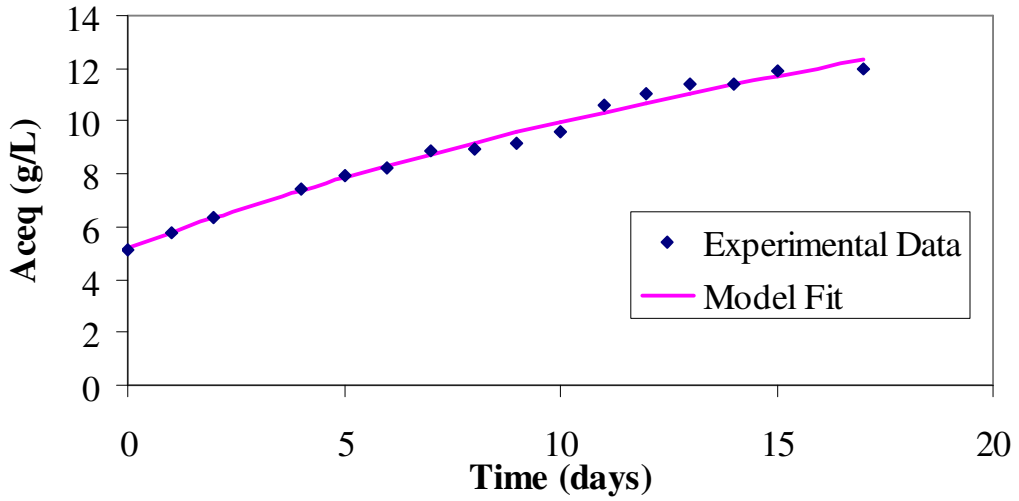


Figure 5-19. Acetic acid equivalent concentration for STW-BM batch fermentation (20 g dry substrate/L of liquid).

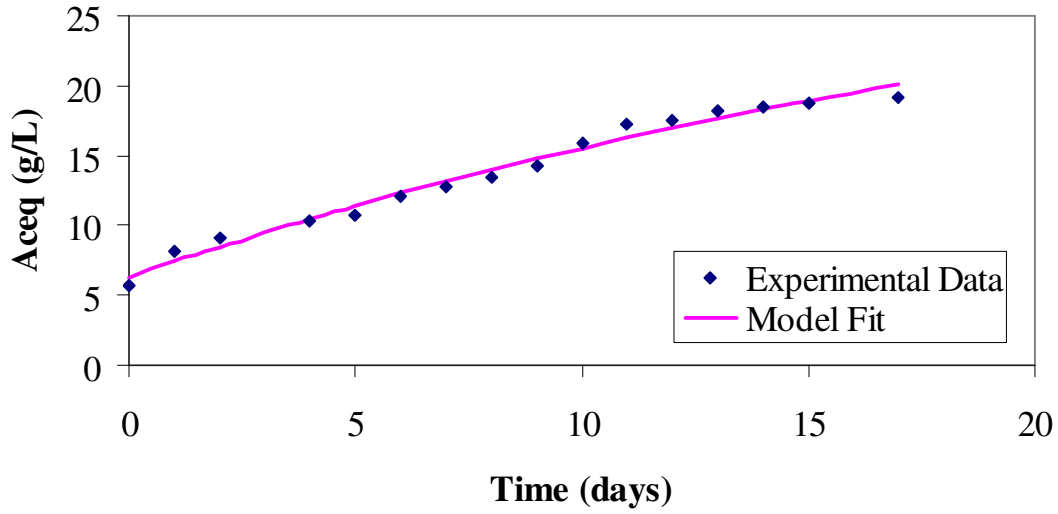


Figure 5-20. Acetic acid equivalent concentration for STW-BM batch fermentation (40 g dry substrate/L of liquid).

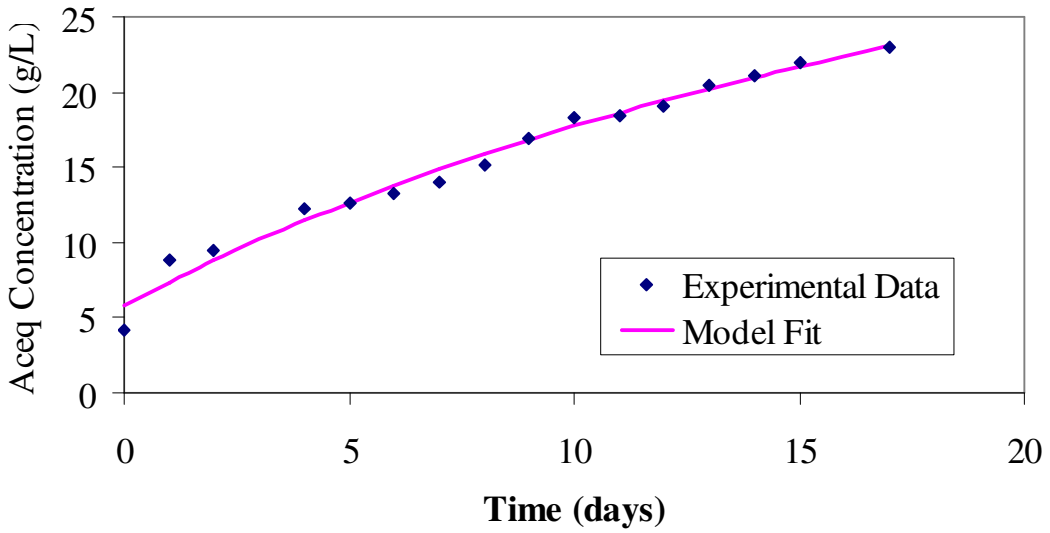


Figure 5-21. Acetic acid equivalent concentration for STW-BM batch fermentation (70 g dry substrate/L of liquid).

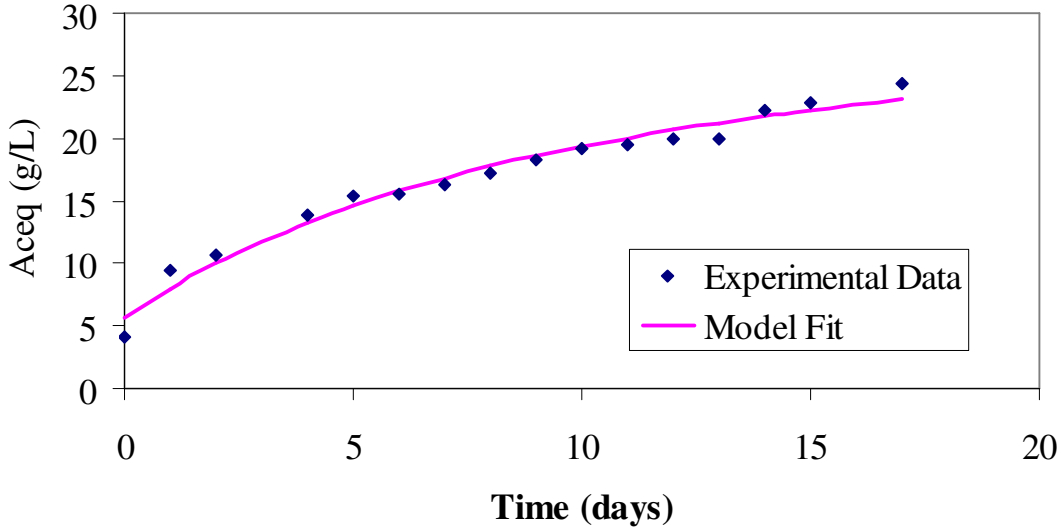


Figure 5-22. Acetic acid equivalent concentration for STW-BM batch fermentation (100 g dry substrate/L of liquid).

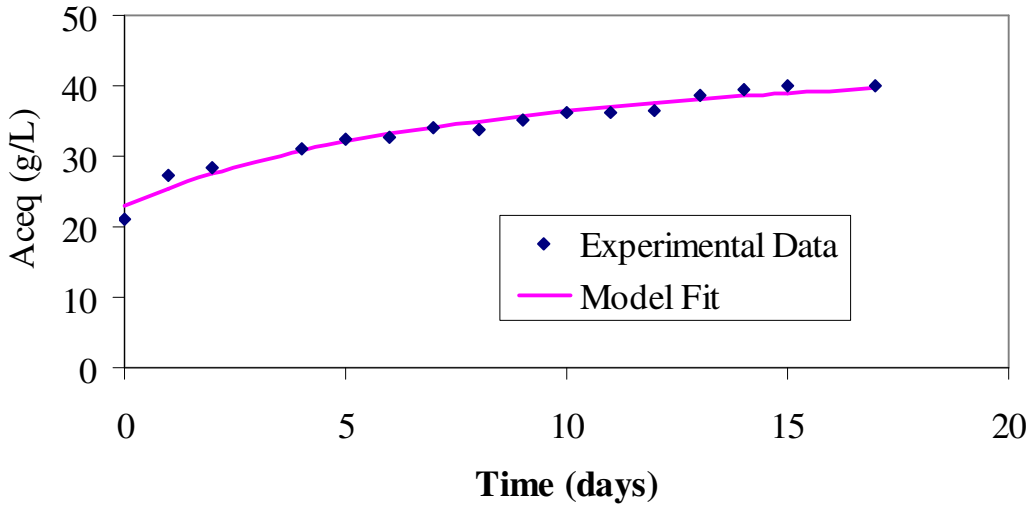


Figure 5-23. Acetic acid equivalent concentration for STW-BM batch fermentation (100^{+} g dry substrate/L of liquid).

Table 5-8. The values of *a*, *b*, and *c* for STW-BM batch fermentation

Initial Substrate Concentration (g/L)	*a* (g/L liquid)	*b* (g /(L liquid·d))	*c* (d^{-1})
20	5.19	0.598	0.025
40	6.27	1.162	0.025
70	5.84	1.588	0.033
100	5.56	2.645	0.092
100^{+}	22.89	2.815	0.108

The predicted specific rate equation is:

$$\hat{r}_{pred} = \frac{0.62(1-x)^{3.08}}{1+4.24(\phi \cdot \text{Aceq})^{0.556}} \qquad (5\text{-}4)$$

Table 5-9. Parameter constant values in CPDM for STW-BM sugarcane trash/chicken manure fermentation with ammonium bicarbonate

Parameter Constant	Short-Term Wash Ball Milled
Holdup (g liquid/g VS cake)	4.27
Moisture (g liquid/g VS fed)	0.05
Selectivity (g Aceq/g VS digested)	0.646
F1–F4 solids concentration (g VS/L)	300
F1–F4 liquid volume (L)	0.21
Φ (g total acid/g Aceq)	0.9
e (g Aceq/(g VS·d))	0.62
f (dimensionless)	3.08
g (L/g total acid)$^{1/h}$	4.24
h (dimensionless)	0.556

Figure 5-24 shows the CPDM "map" for the STW-BM sugarcane trash/chicken manure fermentation system at 300 g VS/L liquid. The "map" predicts a total acid concentration of 61 g/L at LRT of 30 days, VSLR of 7 g/(L·d), and conversion of 55%. High conversion of 72% and high acid concentration of 50 g/L are achieved at a VSLR of 4 g/(L·d) and LRT of 30 days.

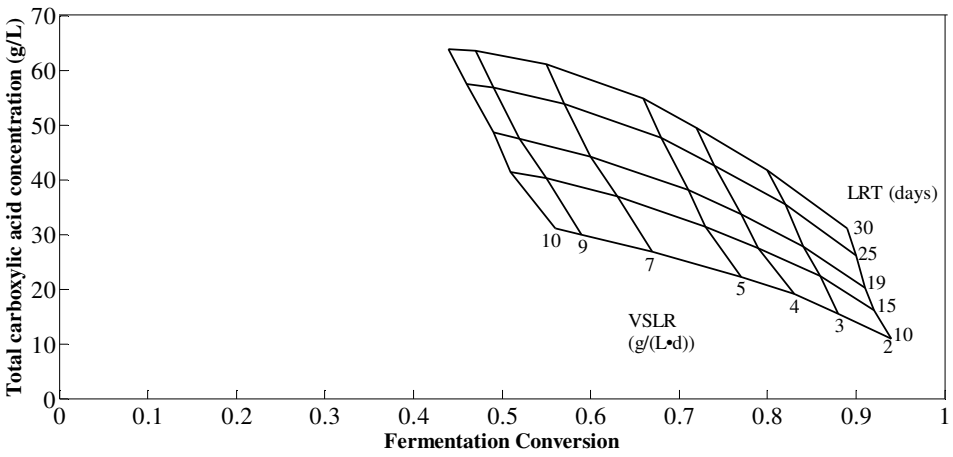


Figure 5-24. CPDM "map" for 80 wt% STW-BM sugarcane trash/20 wt% chicken manure countercurrent fermentation using ammonium bicarbonate buffer at 300 g VS/L of liquid.

5.3.5 CPDM Prediction for STNW-BM

Figures 5-25 to 5-29 show the experimental acid profile and the model fit for the short-term, no-wash, oxidative lime pretreated, and ball-milled (STNW-BM) sugarcane trash/chicken manure batch fermentation. Table 5-10 presents the values of the fitted parameters *a*, *b*, and *c*. The predicted specific reaction rate is shown in Equation 5-5. The CPDM parameters required for the *Mathematica* program are given in Table 5-11.

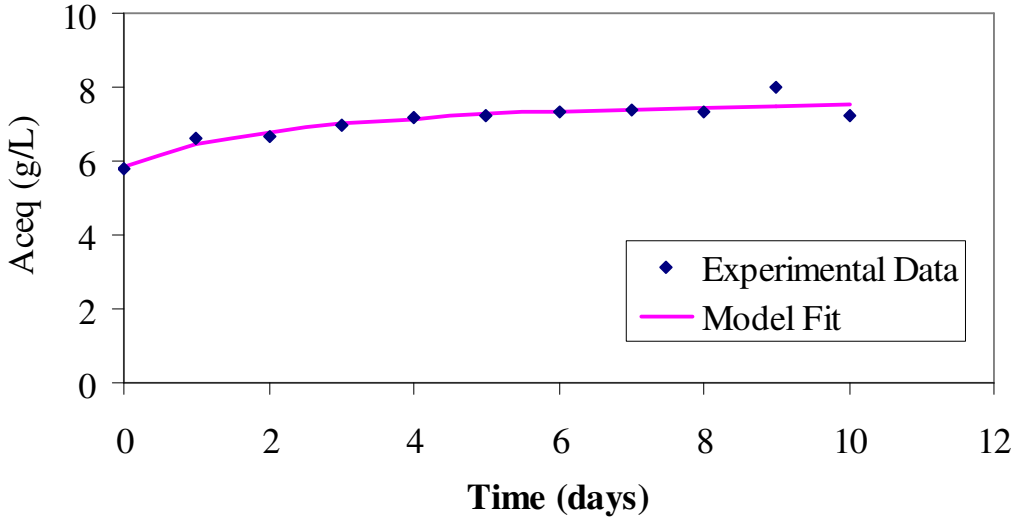


Figure 5-25. Acetic acid equivalent concentration for STNW-BM batch fermentation (20 g dry substrate/L of liquid).

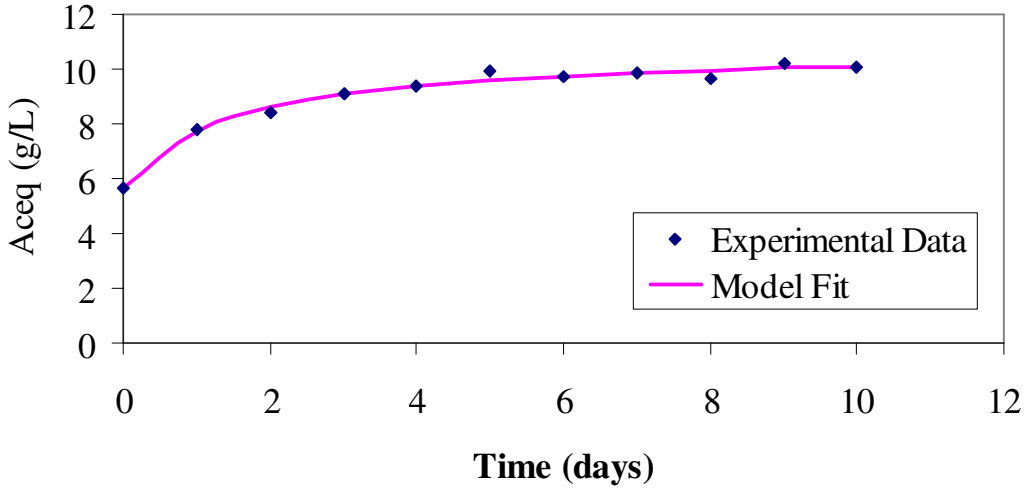


Figure 5-26. Acetic acid equivalent concentration for STNW-BM batch fermentation (40 g dry substrate/L of liquid).

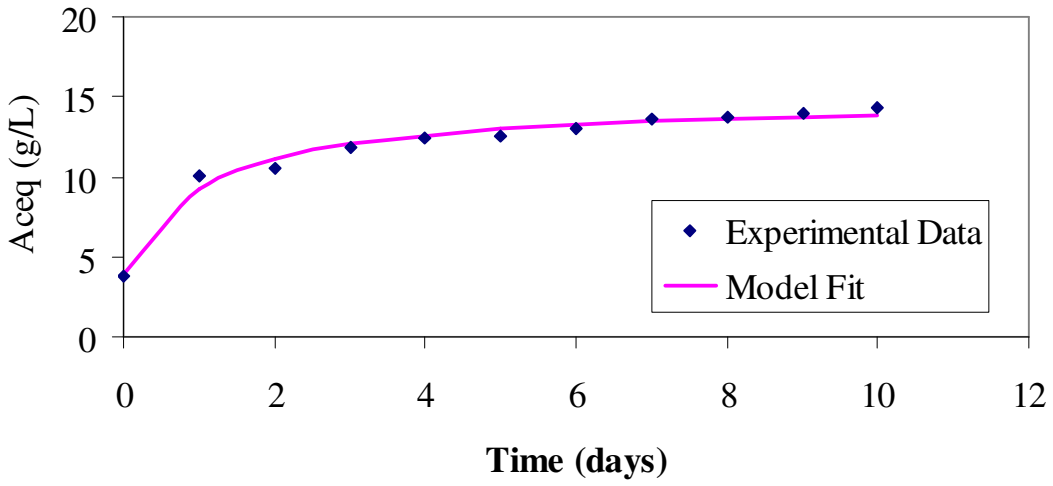


Figure 5-27. Acetic acid equivalent concentration for STNW-BM batch fermentation (70 g dry substrate/L of liquid).

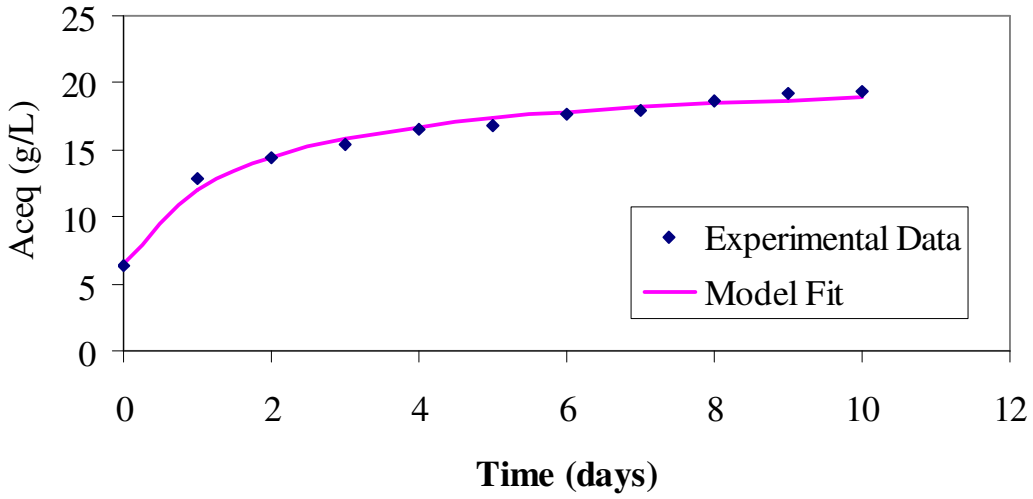


Figure 5-28. Acetic acid equivalent concentration for STNW-BM batch fermentation (100 g dry substrate/L of liquid).

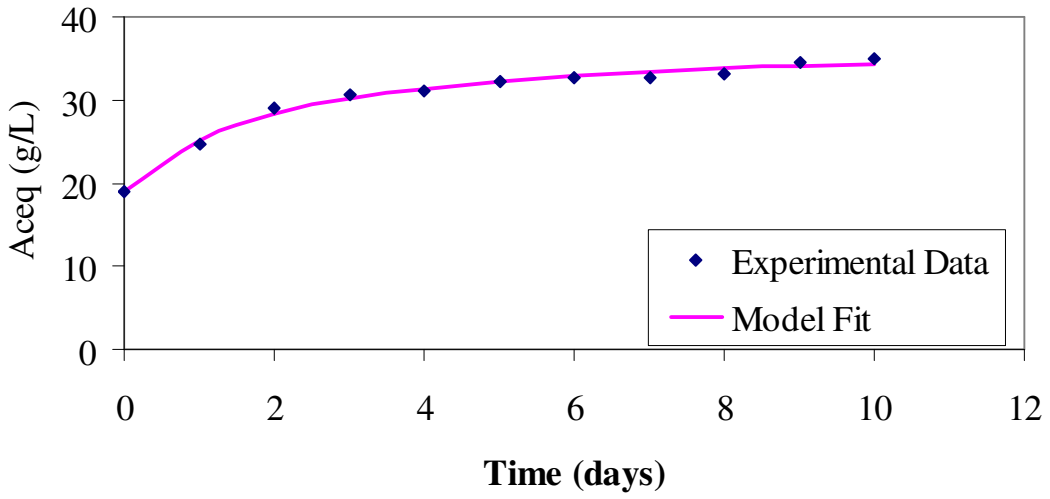


Figure 5-29. Acetic acid equivalent concentration for STNW-BM batch fermentation (100^{+} g dry substrate/L of liquid).

Table 5-10. The values of *a*, *b*, and *c* for STNW-BM batch fermentation

Initial Substrate Concentration (g/L)	*a* (g/L liquid)	*b* (g /(L liquid·d))	*c* (d^{-1})
20	5.83	0.875	0.413
40	5.63	3.593	0.704
70	3.96	10.093	0.918
100	6.52	8.867	0.618
100^{+}	18.99	9.485	0.517

The predicted specific rate equation is:

$$\hat{r}_{pred} = \frac{1.24(1-x)^{3.02}}{1+6.23(\phi \cdot \mathrm{Aceq})^{0.62}} \qquad (5\text{-}5)$$

Table 5-11. Parameter constant values in CPDM for STNW-BM sugarcane trash/chicken manure fermentation with ammonium bicarbonate

Parameter Constant	Short-Term No-Wash Ball Milled
Holdup (g liquid/g VS cake)	4.27
Moisture (g liquid/g VS fed)	0.07
Selectivity (g Aceq/g VS digested)	0.646
F1–F4 solids concentration (g VS/L)	300
F1–F4 liquid volume (L)	0.21
Φ (g total acid/g Aceq)	0.9
e (g Aceq/(g VS·d))	1.24
f (dimensionless)	3.02
g (L/g total acid)$^{1/h}$	6.23
h (dimensionless)	0.62

Figure 5-30 shows the CPDM "map" for the STNW-BM sugarcane trash/chicken manure fermentation system at 300 g VS/L liquid. The "map" predicts a total acid concentration of 64 g/L at LRT of 30 days, VSLR of 7 g/(L·d), and conversion of 57%. High conversion of 76% and high acid concentration of 52 g/L are achieved at a VSLR of 4 g/(L·d) and LRT of 30 days.

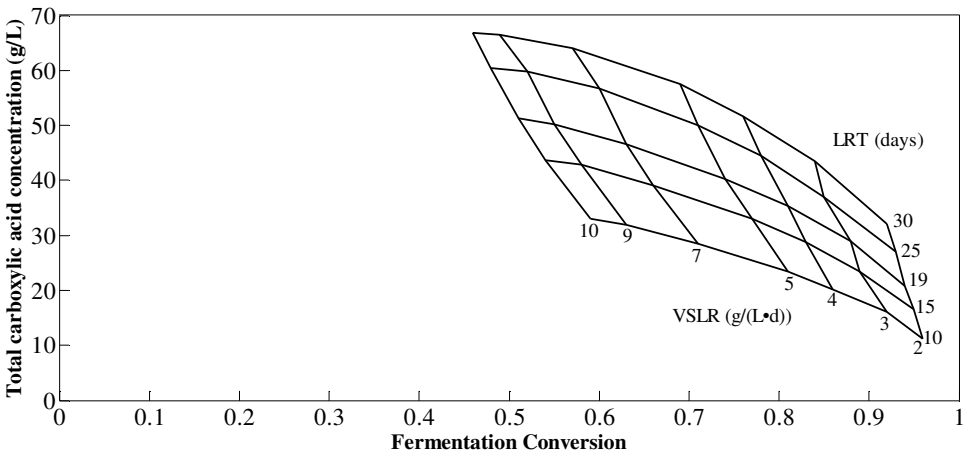


Figure 5-30. CPDM "map" for 80 wt% STNW-BM sugarcane trash/20 wt% chicken manure countercurrent fermentation using ammonium bicarbonate buffer at 300 g VS/L of liquid.

5.4 Effect of Washing

The effect of washing the pretreated biomass on the fermentation performance for the sugarcane trash/chicken manure fermentation system was investigated in this research. After pretreatment, the biomass is neutralized to adjust the pH to between 6.0 and 7.0. Washing the biomass separates the pretreated biomass from the pretreatment liquid (Figure 5-31). The pretreatment liquid contains degraded lignin as well as degraded carbohydrates, particularly the hemicellulose (xylan) fraction of the biomass. Cellulose is better preserved than hemicellulose during pretreatment (Sierra Ramirez, 2005). The pretreatment liquid contains fermentable volatiles and washing removes them. In contrast, if the pretreated biomass is not washed, the wet biomass is air dried for a week without separating the pretreatment liquid. As such, when comparing the

fermentation performance to determine the effect of washing, pretreated biomass that is not washed should perform better.

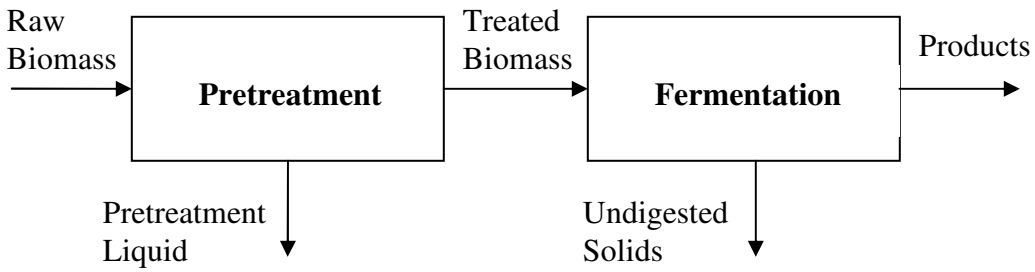


Figure 5-31. Schematic of biomass washing.

Figures 5-32 to 5-34 show the CPDM maps for three different scenarios. In each case the manipulated variable is the washing of the biomass. Figure 5-32 compares the fermentation performance of short-term, acid-washed, oxidative lime pretreated, and ball-milled (STW-BM) sugarcane trash with that of short-term, no-wash, oxidative lime pretreated, and ball-milled (STNW-BM) sugarcane trash. The "map" shows STNW-BM pretreatment has higher acid concentrations and conversions compared to STW-BM. Figure 5-33 compares the fermentation performance of short-term, acid-washed oxidative lime pretreated (STW) sugarcane trash with that of short-term, no-wash, oxidative lime pretreated (STNW) sugarcane trash. Figure 5-33 shows the same trend as observed before with higher acid concentrations and conversions favoring no washing of the pretreated biomass. However, the differences are more subtle in this scenario with

the maps almost overlapping each other. Figure 5-34 compares the fermentation performance of long-term air-lime pile pretreated (LTW) sugarcane trash with that of long-term, submerged, air-lime pretreated (LTNW) sugarcane trash. Figure 5-34 is consistent with the performance predicted by the other two figures. In conclusion, washing the biomass has a negative impact on the fermentation performance, as predicted. At VSLR of 7 g/(L·d) and LRT of 30 days, the predicted increase in acid concentration is about 4–5% (2–3 g/L) and conversions are predicted to rise by 2%. This shows that it is better to just neutralize the biomass and feed the entire contents from the pretreatment unit operation to the anaerobic fermentation unit operation.

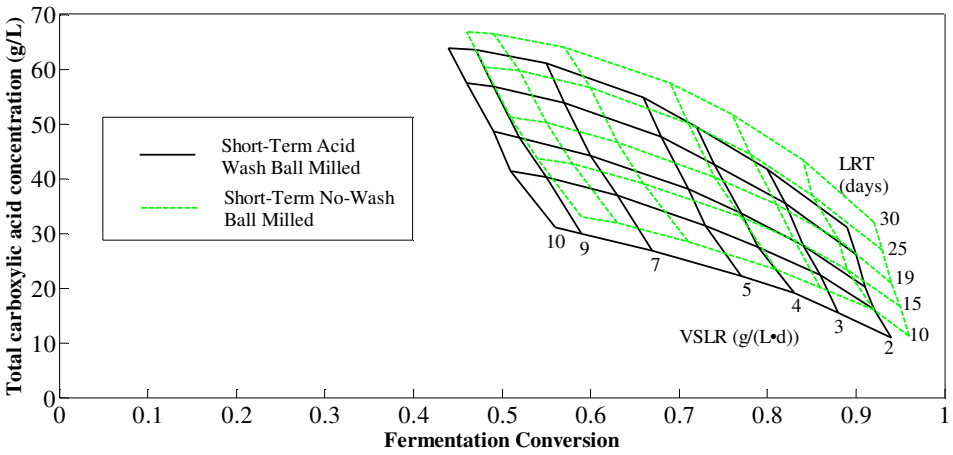


Figure 5-32. CPDM "map" comparing STW-BM and STNW-BM treated 80 wt% sugarcane trash/20 wt% chicken manure countercurrent fermentation using ammonium bicarbonate buffer at 300 g VS/L of liquid.

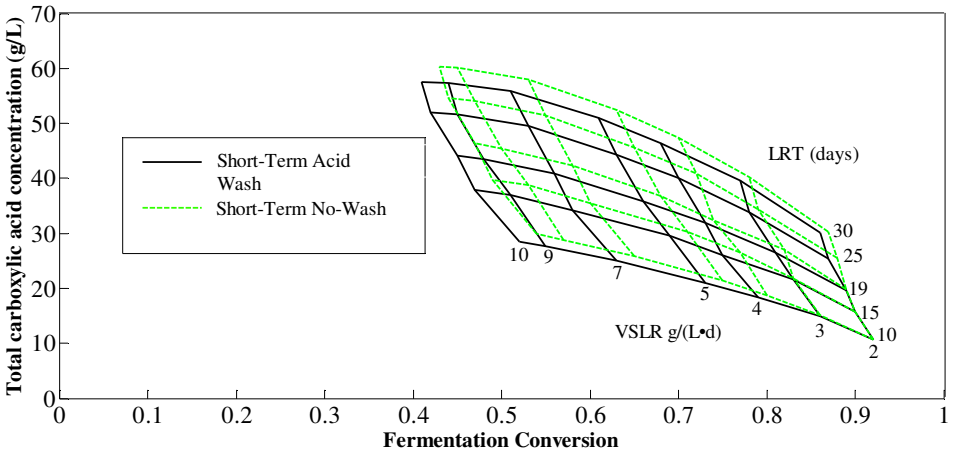


Figure 5-33. CPDM "map" comparing STW and STNW treated 80 wt% sugarcane trash/20 wt% chicken manure countercurrent fermentation using ammonium bicarbonate buffer at 300 g VS/L of liquid.

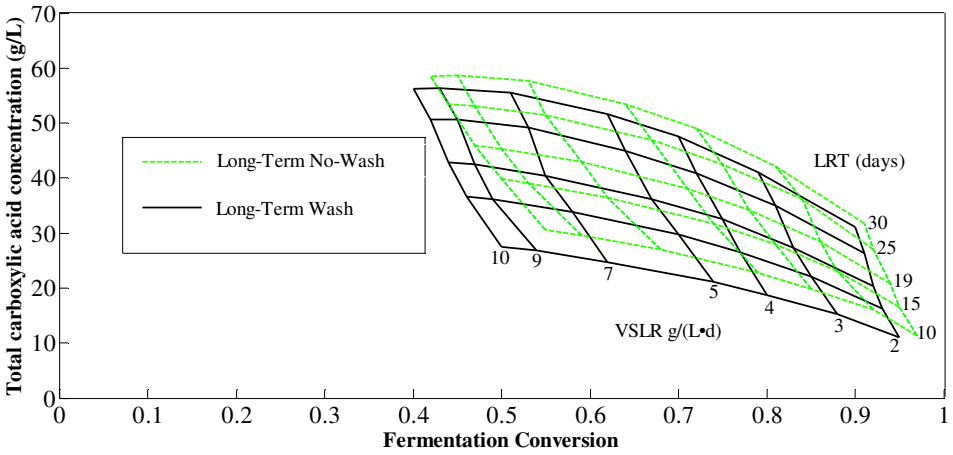


Figure 5-34. CPDM "map" comparing LTNW and LTW treated 80 wt% sugarcane trash/20 wt% chicken manure countercurrent fermentation using ammonium bicarbonate buffer at 300 g VS/L of liquid.

5.5 Effect of Ball Milling

The effect of ball milling on the fermentation performance of the sugarcane trash/chicken manure fermentation system was investigated in this research. Ball milling (Figure 2-7) reduces the crystallinity of the cellulose fraction of the biomass, thereby increasing its digestibility. Ball milling grinds the biomass to a fine powder, which increases the surface area to volume ratio and enables easier access for the microorganisms in the marine inoculum. This results in more efficient utilization of the biomass and increases the acid productivity in anaerobic fermentations. Ball milling was performed for 3 days on all samples.

Figure 5-35 compares the fermentation performance of short-term, acid-washed, oxidative lime pretreated (STW) sugarcane trash with that of short-term, acid-washed, oxidative lime pretreated, and ball-milled (STW-BM) sugarcane trash. The STW-BM sugarcane trash/chicken manure fermentation has higher acid concentration and conversions than the STW sugarcane trash/chicken manure fermentation. Figure 5-36 compares the fermentation performance of short-term, no-wash, oxidative lime pretreated (STNW) sugarcane trash with that of short-term, no-wash, oxidative lime pretreated, and ball-milled (STNW-BM) sugarcane trash. As predicted this figure also shows a significant increase in acid concentrations and conversions associated with ball milling. In conclusion, ball milling has a positive impact on the fermentation performance of sugarcane trash/chicken manure fermentation system. At VSLR of 7 g/(L·d) and LRT of 30 days, the predicted increase in acid concentration is about 9–10% (5–6 g/L) and conversions are predicted to rise by 4%.

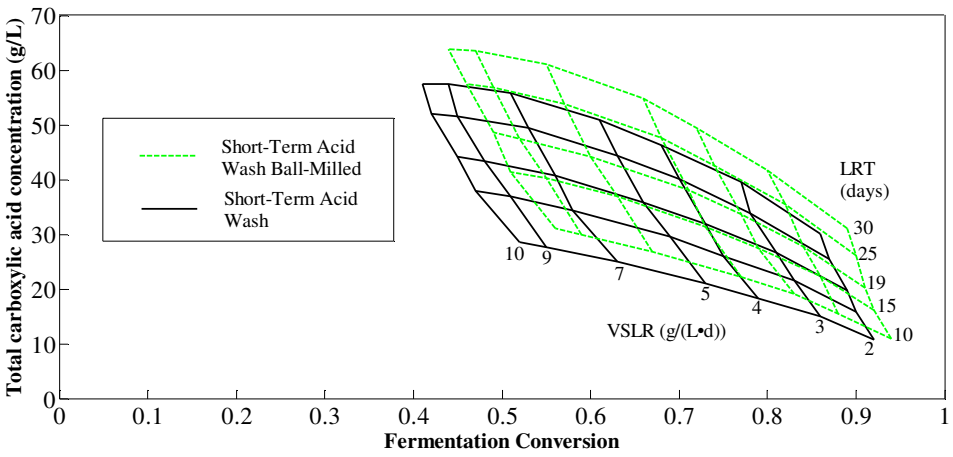


Figure 5-35. CPDM "map" comparing STW-BM and STW treated 80 wt% sugarcane trash/20 wt% chicken manure countercurrent fermentation using ammonium bicarbonate buffer at 300 g VS/L of liquid.

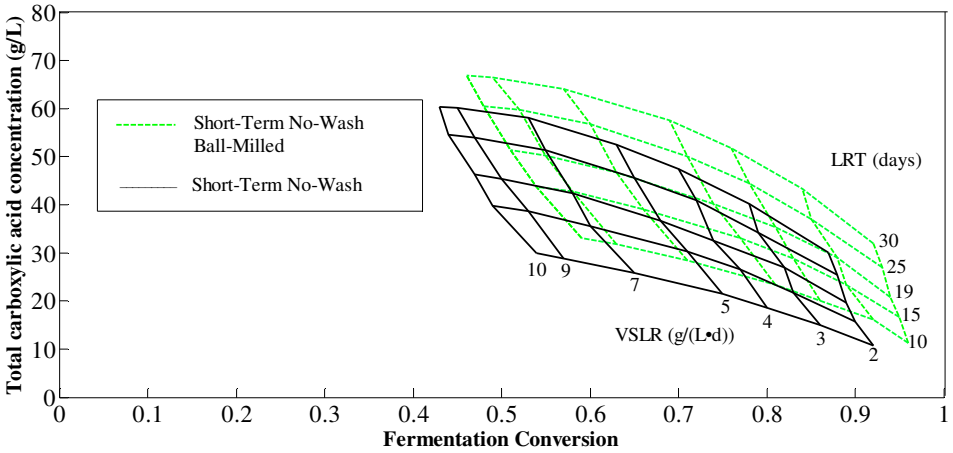


Figure 5-36. CPDM "map" comparing STNW-BM and STNW treated 80 wt% sugarcane trash/20 wt% chicken manure countercurrent fermentation using ammonium bicarbonate buffer at 300 g VS/L of liquid.

5.6 Effect of Pretreatment Conditions

In this section evaluates two different pretreatment conditions are evaluated: short-term oxidative lime pretreatment at 110°C and 0.791 MPa (100 psig) oxygen pressure (Appendix C) and long-term air-lime pretreatment at 55°C performed for 5 weeks (Appendix A and B). The purpose was to determine which is a better option for sugarcane trash.

Figure 5-37 compares the fermentation performance of short-term, acid-washed, oxidative lime pretreated (STW) sugarcane trash with that of long-term air-lime pile pretreated (LTW) sugarcane trash. At low VSLR, the LTW fermentation has higher conversions and acid concentrations, but as the VSLR increases the fermentation performance for both pretreatment conditions is almost identical. Figure 5-38 compares the fermentation performance of short-term, no-wash, oxidative lime pretreated (STNW) sugarcane trash with that of long-term, submerged, air-lime pretreated (LTNW) sugarcane trash. Similar to Figure 5-37, at low VSLR, the LTNW fermentation is better than the STNW, but as the VSLR increases, the differences are too close to favor one pretreatment condition over the other.

In conclusion, short-term oxidative lime pretreatments and long-term air-lime treatments show only slight differences in fermentation performance when the CPDM method is applied. Long-term air-lime pretreatments result in higher acid concentrations at low VSLR and at high conversions. However, the time required for long-term pretreatments (~2 months) compared to just few hours for short-term pretreatments can

be a decisive factor. Also using pure oxygen to maintain oxidative conditions can be expensive.

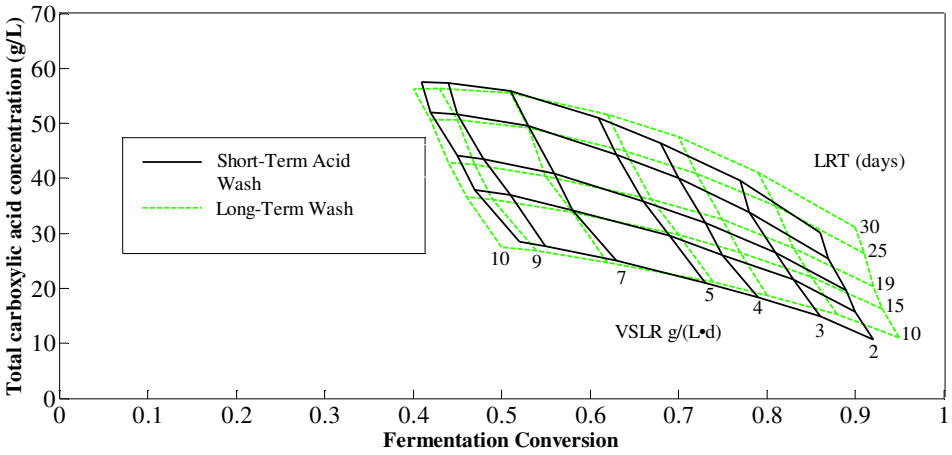


Figure 5-37. CPDM "map" comparing STW and LTW treated 80 wt% sugarcane trash/20 wt% chicken manure countercurrent fermentation using ammonium bicarbonate buffer at 300 g VS/L of liquid.

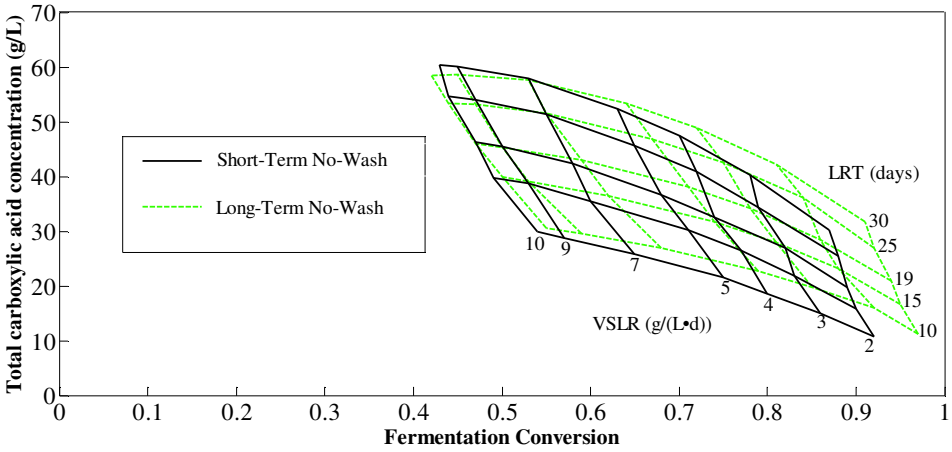


Figure 5-38. CPDM "map" comparing STNW and LTNW treated 80 wt% sugarcane trash/20 wt% chicken manure countercurrent fermentation using ammonium bicarbonate buffer at 300 g VS/L of liquid.

5.7 Conclusions

In this chapter, several different pretreatment methods shown on Table 5-1 were investigated to determine the best pretreatment method for the sugarcane trash substrate used in this research. CPDM was used to generate maps that predict the acid concentrations and conversions at various VSLR and LRT. Based on the predictions, the best-performing pretreatment method was the short-term, no-wash, oxidative lime pretreatment with ball-milling (STNW-BM). The CPDM method predicts acid concentrations as high as 64 g/L and 57% conversion at VSLR of 7 g/(L·d) and LRT of 30 days. This acid concentration is about 15% higher than that predicted for the LTW sugarcane trash presented in Chapter IV and also 3% higher than that predicted for the air-lime pretreated bagasse at the same VSLR and LRT. Also high conversion of 76% and high acid concentration of 52 g/L are achieved at a VSLR of 4 g/(L·d) and LRT of 30 days.

The effect of three manipulated variables was evaluated individually in this chapter (i.e., washing biomass, ball milling, and pretreatment condition). It was determined that washing separates the pretreatment liquid from the treated biomass. Thus, washing decreases the acid concentration and conversions because the pretreatment liquid contains fermentable solubles. Ball milling had a positive and the most significant impact among the variables investigated. Ball milling reduces the crystallinity of the cellulose fraction of the biomass and increases the digestibility. At VSLR of 7 g/(L·d) and LRT of 30 days, the predicted increase in acid concentration is about 9–10% (5–6 g/L) and conversions are predicted to rise by 4%. Short-term

oxidative conditions were compared against long-term air-lime treatment conditions. As low VSLR, the long-term air-lime-treated sugarcane trash fermentations had higher conversions and acid concentrations, but as the VSLR increased, there was very little difference between the two pretreatment conditions.

Result of countercurrent experiments presented in Chapter IV deemed sugarcane trash to be a slightly inferior substrate choice compared to sugarcane bagasse in the MixAlco process. This chapter proves that with the proper pretreatment method sugarcane trash is a very attractive feedstock for the MixAlco process. A summary of the peak acid concentration and the corresponding conversions is presented in Table 5-12.

Table 5-12. Results of CPDM prediction at VSLR 7 g/(L·d) and LRT 30 days

Feedstock type (four-stage countercurrent fermentation, 300 g VS/L liquid)	CPDM predicted peak acid concentration (g/L)	Predicted conversion
Air-lime-treated bagasse	62.4	0.51
Air-lime-treated sugarcane trash		
Long-term wash	55.6	0.51
Long-term no-wash	57.6	0.53
Short-term wash	55.8	0.51
Short-term wash ball-milled	61.0	0.55
Short-term no-wash	58.0	0.53
Short-term no-wash ball-milled	64.0	0.57

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

This research work investigated the feasibility of using sugarcane trash as a feedstock for the MixAlco process. Preliminary batch studies showed sugarcane trash to be more productive than the control, which was copier paper. Over a 20-day period, sugarcane trash batch experiments produced an average of 19.7 g/L of carboxylic acids. The batch experiments showed the reactivity to be very similar to sugarcane baggase. Sugarcane trash had the highest average yield (0.31 g total acid/g VS digested) and highest average conversion (0.70 g VS digested/g VS fed) among the three substrates compared in the batch experiments. Batch experiments also showed that ammonium bicarbonate buffered fermentations could produce 85–90% acetate content, which would be highly desirable if the end product is ethanol in the MixAlco process.

Countercurrent experiments at three different VSLR and LRT were also performed in the laboratory. These experiments took about 3 months to reach steady state. Data were collected for almost another 2 months after reaching steady state to quantify the fermentation performance and to check mass balance closure. Fermentation Train C (VSLR = 4.58 g/(L·d) and LRT = 25 days) with a carboxylic acid concentration of 29.9 g/L had the highest acid productivity of 1.40 g total acid/(L liquid·d). Fermentation Train A (VSLR = 3.49 g/(L·d) and LRT = 21 days) with a carboxylic acid concentration of 26.3 g/L had the highest conversion (0.64 g VS digested/g VS fed), yield (0.36 g total acid/g VS fed), and selectivity (0.65 g Aceq/g VS digested). As the VSLR increased, conversions and yield decreased because only a fraction of the total

biomass fed was digested. However, as the VSLR increased, the acid productivity and concentrations increased because more reactive solids were being digested by the microorganisms. At low VSLR, the acid productivity is low because there is less biomass, but the conversion and yield is higher because the microorganisms had time to digest both the reactive and recalcitrant portions of the biomass. The selectivity did not show any clear trend. Fermentation Train A had the highest mass balance closure of 91%. Possible reasons for closures not being 100% include errors in measurement (e.g., gas volume and liquid volume) and other discrepancies in the transfer procedure.

The validity of the CPDM model was evaluated by establishing batch experiments at various initial solids concentrations. The experimental specific reaction rates obtained were fit to an empirical equation with four parameters *e, f*, *g*, and *h* using non-linear regression. These parameters and others (holdup, selectivity, ratio of acids to acetic acid equivalents, liquid volume, and moisture) were used in the CPDM *Mathematica* program to predict the acid concentrations and conversions at conditions resembling Trains A to C. The average error between the predicted and experimental carboxylic acid concentration was 4.62%. The average error between the predicted and experimental conversions was 1.42%. The highest error in total carboxylic acids was 8.77% and the highest error in conversion was 2.91%. The CPDM "map" at 300 g VS/L was generated for the air-lime pile pretreated sugarcane trash/chicken manure fermentation system used in the countercurrent experiments. This high volatile solid concentration was selected to best resemble an industrial-scale fermentor to determine the commercial feasibility of the process. The "map" predicted a total acid concentration

of 55.6 g/L at LRT of 30 days, VSLR of 7 g/(L·d), and conversion of 51%. Fermentation conversion of 70% and high acid concentration of 47.5 g/L are predicted at a VSLR of 4 g/(L·d) and LRT of 30 days. In contrast, sugarcane bagasse/chicken manure fermentation predicted a total acid concentration of 62.4 g/L at LRT of 30 days, VSLR of 7 g/(L·d), and conversion of 51%.

Several different pretreatment methods were also investigated to determine the best pretreatment method for sugarcane trash. Instead of using enzymatic hydrolysis to determine the effectiveness of various pretreatments performed, CPDM was used to compare the maps at 300 g VS/L and determine the best conditions. Among the pretreatments performed, the best-performing pretreatment method was the short-term, no-wash, oxidative lime pretreatment with ball-milling (STNW-BM). The CPDM "map" predicted a total acid concentration of 64 g/L at LRT of 30 days, VSLR of 7 g/(L·d), and conversion of 57%. This acid concentration is about 15% higher than that predicted for the sugarcane trash used in the countercurrent experiments presented in Chapter IV and also 3% higher than that predicted for the air-lime pretreated bagasse at the same VSLR and LRT. Also, high conversion of 76% and high acid concentration of 52 g/L were achieved at a VSLR of 4 g/(L·d) and LRT of 30 days.

The effect of three manipulated variables was evaluated individually in this chapter (i.e., washing biomass, ball milling, and pretreatment condition). Ball milling had the most significant impact on the fermentation performance of the sugarcane trash/chicken manure system. At VSLR of 7 g/(L·d) and LRT of 30 days, the predicted

increase in acid concentration is about 9–10% (5–6 g/L) and conversions were predicted to rise by 4%.

The results of these studies show that sugarcane trash is a valuable feedstock for the MixAlco process. This study also shows the importance of the pretreatment step to make the biomass more digestible. Short-term pretreatments can save a lot of time, and when combined with ball milling, it can greatly increase the acid productivity of the biomass substrate. In this research, a predetermined temperature of 110°C and oxygen pressure of 0.791 MPa (100 psig) were used for the short-term pretreatments. In the future, several different temperatures ranging from 100 to 180°C and oxygen pressures ranging from 0.791 to 2.86 MPa (100–400 psig) could be investigated. A model fit for the results can be obtained to determine the optimum condition. The lime consumption can also be determined to avoid loading excess amounts of lime. Another idea that could be researched is to use sugarcane trash as a supplemental feedstock for the MixAlco process. It can be combined with other substrates, such as sugarcane bagasse or even sludge, which is a byproduct of wastewater treatment.

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APPENDIX A

LONG-TERM AIR-LIME PILE TREATMENT PROCEDURE

Sugarcane trash (4–5 kg dry weight) was mixed with calcium hydroxide and placed on top of a rock bed in a large plastic storage bin (L × W × H = 3 ft × 2 ft × 2 ft). The water was continuously distributed through the biomass by a water sprayer above the pile, and was recycled through a water heater. A heat exchanger maintained the biomass treatment system at a constant temperature of 50°C. Air was scrubbed through the lime slurry container and then bubbled through the pile via air diffusers beneath the pile.

Procedure

1. Mix a large amount of biomass with excess lime (0.4 g $Ca(OH)_2$/g dry biomass) in a large tub.
2. Form a pile on top of the rock bed with the biomass and lime mixture.
3. Place the dome covering on top of the storage bin.
4. Screw in the unions connecting the inlet and outlet pipes of the sump.
5. Fill the sump with water to about ¾ the height of the bin.
6. Fill the water heater tank with distilled water.
7. Control air flows to diffusers located beneath the pile and maintain the flowrate at 20 standard cubic feet per minute.
8. Make sure the return line valve to the sump is open, and the valve controlling flow the water sprayers is initially closed.
9. Prime both centrifugal pumps.

10. Turn on pumps and allow time for air bubbles to be pushed out of the system. This could take a few minutes.
11. Turn on the water heater.
12. Turn on the temperature controller and set temperature to 50°C.
13. Open and adjust the sprayer valves to the appropriate position to be sure water is being discharged from each sprinkler onto the pile.
14. Monitor sump water level and add more water when required to maintain a constant water level.
15. Monitor the pH of the lime slurry to ensure basic conditions are maintained.
16. Check the system daily for leaks and monitor the strainer in the sump pump discharge line weekly to be sure it is not clogged.
17. The pretreatment can be stopped when the lignin content is reduced by 50% or the pH drops below 9.
18. Pretreatment is usually performed for 5–8 weeks.
19. At the end of pretreatment collect biomass and neutralize using desired method.
20. Flush the system thoroughly with fresh water before using the system again. This may need 6–7 complete flush procedures.

APPENDIX B

LONG-TERM SUBMERGED AIR-LIME TREATMENT PROCEDURE

In the submerged version of the long-term air-lime treatment, approximately 5 kg of sugarcane trash is mixed with calcium hydroxide and transferred to a large cylindrical pretreatment vessel. Distilled water is added to submerge the biomass mixture. The temperature is maintained at 50°C by circulating heated water in the storage tank through the jacketed vessel. Air was scrubbed through the lime slurry container and then bubbled through the air hose placed below the base sieve plate in the cylindrical vessel.

Procedure

1. Measure desired amount of biomass to be pretreated in a large tub.
2. Measure required amount of lime to be added (0.4 g $Ca(OH)_2$/g dry biomass).
3. Wearing gloves, mix biomass and lime well in the tub.
4. Add the mixture to the cylindrical pretreatment vessel and fill vessel with enough distilled water to submerge the biomass/lime mixture.
5. Fill process water tank with water and switch on both pump and temperature controller.
6. Ensure that pump is pumping the water to the jacketed cylindrical vessel. If not, prime the pump and check again.
7. Connect the air lines and adjust air flow through the lime slurry vessel.
8. Stir the mixture in the pretreatment vessel using a long metal stirrer.
9. Cover the vessel and place a thermometer in the vessel to confirm the temperature is at the setpoint of 50°C. If not, adjust the temperature controller.

10. Routinely check the liquid levels in the pretreatment vessel and the hot water tank and top up when necessary to replace the evaporated liquid.
11. Monitor pH of the mixture to maintain basic conditions and check for leaks daily.
12. Stop pretreatment after 5–8 weeks or after pH drops to 9.
13. Neutralize and dry pretreated biomass using the desired method.

APPENDIX C

SHORT-TERM OXIDATIVE LIME TREATMENT PROCEDURE

Short-term oxidative lime treatment was performed in six 304 stainless steel pipe nipples with a 145-mL volume. Biomass (8 g on dry basis), distilled water (120 mL), and calcium hydroxide (3.2 g) was added the reactor and mixed well. All pretreatments were performed at 110°C and a constant oxygen pressure of 0.791 MPa (100 psig) was maintained throughout the 2 hours required for the pretreatment.

Procedure

1. Measure 8 g of biomass and 3.2 g of $Ca(OH)_2$ for each reactor on weighing dishes.
2. Roll 3–4 rounds of Teflon tape on to the uncapped end of each reactor.
3. Screw on 0.25-in Swagelok plug on to the elbow fitting at the capped end of each reactor to prevent leakage when loading.
4. Preheat oven to desired temperature.
5. Empty measured amounts of biomass and lime into reactor.
6. Add 120 mL of distilled water using a manual burette.
7. Hand-tighten stainless steel cap on to the reactor.
8. Tightly seal the reactor using a wrench on the workbench and mix contents well.
9. Invert and slowly remove the 0.25-in plug screwed on the elbow fitting.
10. Attach reactor to the correct position on the manifold by screwing on the plug and tighten using a hand wrench.
11. Perform Steps 5–11 for each reactor.

12. Ensure all the connectors on the manifold are secure and tight.
13. Place manifold assembly in the oven and screw on the flexible hose to the manifold.
14. Close oven door and switch on motor to initiate swinging motion.
15. Turn on oxygen supply and adjust the regulator to ensure oxygen is being supplied at the desired pressure.
16. Open oven door and ensure there are no leakages and immediately close door. Wait for temperature to reach setpoint before starting the timer.
17. At the end of pretreatment, switch off the motor and open the oven door.
18. Turn off oxygen supply and adjust the regulator.
19. Wear gloves and slowly unscrew the flexible metal hose attached to the tubing assembly directly on top of the oven. Steam will be slowly released as the pressure is dropped. Take time to ensure this is done slowly as it can be extremely dangerous because of the high pressure in the reactors.
20. Once it is safe, unscrew the flexible hose and remove the manifold assembly.
21. Quench the assembly in a deep large tray filled with a mixture of ice and water.
22. After reactors have cooled to room temperature, unscrew each one from manifold and screw on the 0.25-in Swagelok plug.
23. Unscrew reactor cap using wrench on the workbench, empty contents into a 1-L centrifuge bottle, and repeat to unload all the reactors.
24. Flush all the reactors and the manifold with distilled water to remove clogged biomass and lime. If needed, replace the connectors when worn.

APPENDIX D

LIQUID MEDIA PREPARATION

Deoxygenated water containing sodium sulfide and cysteine hydrochloride was the liquid medium used in all the fermentation experiments.

Procedure

1. Pour 5 L of distilled water into a large glass container (6-L total volume).
2. Boil distilled water under a nitrogen purge for 5 min.
3. Seal lid with plastic wrap and allow the boiled water to cool to room temperature.
4. Add 0.275 g cysteine hydrochloride and 0.275 g sodium sulfide per liter of the boiled distilled water.
5. Stir the solution and pour into storage bottles with a nitrogen purge. Be sure to fill the bottles completely and close the lid tightly.

APPENDIX E

SINGLE-CENTRIFUGE COUNTERCURRENT TRANSFER PROCEDURE

In countercurrent fermentations, liquid and solids flow in opposite directions in a train of four fermentors. In the laboratory, the transfer of liquid and solids is made every 2 days, resembling more of a semi-continuous operation. Countercurrent fermentations were initiated as batch fermentations. The experiments were performed in batch mode for 2 weeks until the culture was established in the fermentor. Countercurrent transfers are started after 2 weeks and the liquids and solids were transferred using the single-centrifuge procedure (Figure E-1). All the collected data were recorded in data sheets similar to Table E-1. To maintain anaerobic conditions in the fermentors, a nitrogen purge should be utilized every time the fermentors are open to the atmosphere.

Single-Centrifuge Procedure

1. Remove all the fermentors from the incubator and allow cooling to room temperature for 10 minutes.
2. Use syringes to draw 3-mL gas samples from the fermentors if analyzing for gas composition. If not, vent and record gas production using the device illustrated in Figure 2-13.
3. Remove the fermentor caps one at a time and place a nitrogen purge line in the fermentor. Using another nitrogen line, clear the residual solids adhered to the stopper and metal bars back into the fermentor.
4. Measure and record the pH for each fermentor.
5. Cap the fermentor with a regular centrifuge cap.

6. Once the pH of all fermentors has been recorded, arrange the fermentors in terms of descending weight and balance each pair of fermentors with weight supplements (pre-weighed cardboard or metal piece).
7. Centrifuge the fermentors to separate the solid and the liquid. Centrifuge time varies with the susbstrate systems. A time of 25 minutes was used for the sugarcane trash/chicken manure system. The speed was at 4000 rpm and brake level was set to 5.
8. After centrifuging, handle the bottles carefully with as little sudden movement as possible to ensure that the solids and liquid do not remix.
9. Pour the liquid from Fermentor 1 (F1 in Figure E-1) into previously weighed plastic graduate cylinder. Record the weight and volume of the product liquid.
10. Take a 3-mL liquid sample for carboxylic acid analysis. Decant the remaining liquid from F1 into a liquid collection bottle for further VS analysis. Have a separate collection bottle for each fermentor train and store the bottle in a freezer until VS analysis is performed.
11. Weigh the fermentor with the remaining wet cake and compare against the goal weight. Remember to remove the centrifuge cap when the weight is measured. The kept solid weight for the first bottle will be equal to: Target weight (300 g) minus solid loading for that train. The target weight of 300 g was selected for the sugarcane trash/chicken manure fermentation system. The kept solid weight will be equal to the target weight in Fermentors 2–4.

Example:

Weight of F1 + wet solid cake = 320 g

Biomass loading for train = 10 g

Kept solid weight = 300 g (Target weight) – 10 g (Biomass loading) = 290 g

Removed solid weight = 320 g – 290 g = 30 g

12. Add fresh biomass to F1 according to the determined loading rate.
13. Pour all the liquid from F2 into F1.
14. Add methane inhibitor, mix well, and record the pH after transfer.
15. Add ammonium bicarbonate if required to control the pH at the desired range of 7.00–7.25. Record the adjusted pH.
16. Replace the stopper and cap the fermentor.
17. Weigh the wet solids from F2. Remove the excess solids and keep aside to be added to F3.

Example:

Removed solid weight from F1 to be added to F2 = 30 g

Weight of F2 + wet solid cake = 285 g

Kept solid weight = Target weight = 300 g

Removed solid weight = F2 solids weight + F1 excess – target weight

Removed solid weight = 285 g + 30 g – 300 g = 15 g (F2 excess)

18. Add the excess solid from F1 into F2.
19. Pour all the liquid from Fermentor 3 (F3 in Figure E-1) into F2, and repeat Steps 14–16.
20. Determine and remove the excess solid weight from Fermentor 3 (F3 in Figure E-1) and add the excess solid from F2 into F3.
21. Pour all the liquid from Fermentor 4 (F4 in Figure E-1) into F3, and repeat Steps 14–16.
22. Determine and remove the excess solid from F4 and store in solid collection bottle for later VS analysis. Have a separate collection bottle for each fermentor train and store the bottle in a freezer until VS analysis is performed.
23. Add 100 mL of fresh liquid medium to F4 and repeat Steps 14–16.
24. Return all fermentors back to the incubator.

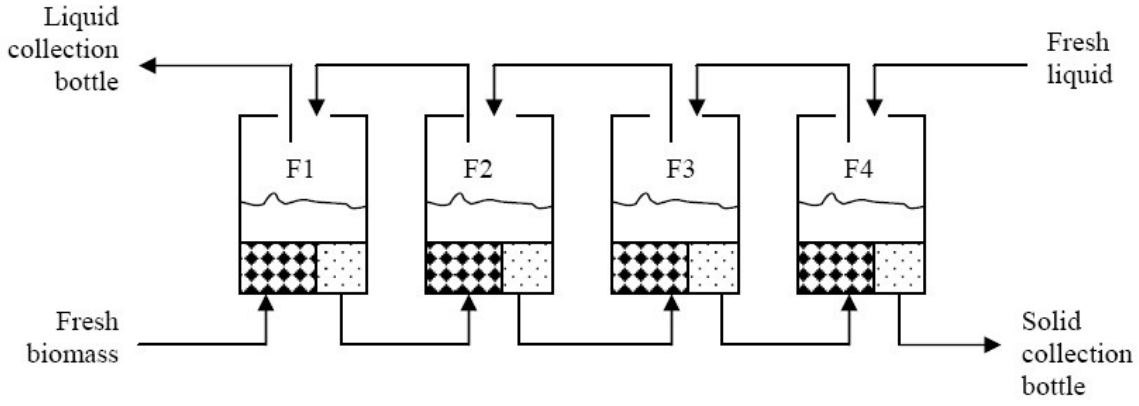

Figure E-1. Single-centrifuge procedure.

Table E-1. Countercurrent fermentations data sheet

	Gas Initial Position	Gas Final Position	Initial pH	pH after transfer	pH adjusted	Liquid Weight (g)	Liquid Volume (ml)	Solid Weight (g)	Kept Solid Weight (g)	Removed Solid Weight (g)	Added Solid Weight (g)	Added Liquid Volume
A1									290		8 g Substrate + 2 g Chicken Manure	
A2									300			
A3									300			
A4									300			Fresh 100 mL
B1									288		9.6 g Substrate + 2.4 g Chicken Manure	
B2									300			
B3									300			
B4									300			Fresh 100 mL
C1									286		11.2 g Substrate + 2.8 g Chicken Manure	
C2									300			
C3									300			
C4									300			Fresh 100 mL

APPENDIX F

CARBOXYLIC ACIDS ANALYSIS

For carboxylic acids analysis, at least 3 mL of liquid sample should be withdrawn from the fermentor, and placed in a 15-mL conical-bottom centrifuge tube. If the samples are not analyzed immediately, they should be stored in a freezer at –15°C. At the moment of analysis, if the samples were stored in the freezer, defrost and vortex the sample before beginning the procedure. If the acid concentration is high, the sample might require to be diluted with water (50 vol% sample/50 vol% water).

GC Liquid Sample Preparation

1. Centrifuge the liquid samples in the centrifuge tubes for 10 min at 4000 rpm.
2. Pipette 1 mL of the clear liquid broth into a 15-mL round bottom ultracentrifuge tube.
3. Add to the same tube 1 mL of 10-mM of internal standard 4-methyl-varleric acid (1.1.62 g/L internal standard, ISTD).
4. Add to the same tube, 1 mL of 3-M phosphoric acid to acidify the sample and allow the carboxylic acids to be released in the GC injection port.
5. Cap the tube and vortex.
6. Centrifuge the mixture at 15,000 rpm in the IEC B-20A centrifuge machine (Industrial Equipment Co., Needham Hts., MA). Set the mode of centrifuge machine as refrigeration mode until the temperature inside the centrifuge machine is lower than 25°C. Because of the poor refrigeration system in this centrifuge machine,

simply accelerate the centrifuge rotating speed to 15,000 rpm and immediately turn the knob back to zero rpm.

7. Remove the round-bottom ultracentrifuge tubes and pipette 1 mL of the mixture into a glass GC vial. The centrifuged sample in the vial is ready to be analyzed now.
8. If the prepared sample will not be analyzed immediately, it can be stored in the freezer. If frozen, care should be taken to thaw and vortex the sample before the GC analysis.

GC Operation

1. Before starting the GC, check the gas supply cylinders (compressed hydrogen, compressed zero-grade helium, and compressed zero-grade air from Praxair Co., Bryan, TX) to insure at least 100 psig pressure in each gas cylinder. If there is not enough gas, switch cylinders and place an order for new ones.

2. Regulate gas flow by setting the regulators in 40 psig for hydrogen, 60 psig for helium, and 50 psig for air.

3. Check the solvent and waste bottles on the injection tower. Fill up the solvent bottles with methanol around neck level. Empty the waste bottles.

4. Make sure the column head pressure gauge on the GC indicates the proper pressure (15 psig). Low head pressure usually indicates a worn-out septum. Replace the septum before starting the GC.

5. Up to 100 samples can be loaded in the autosampler plate in one analysis batch. Place the samples in the autosampler racks, not leaving empty spaces between samples. Place

volatile acid standard mix (Matreya Inc., Catalog # 1075) solution every 50 samples for calibration.

6. Check the setting conditions in the method:

a. Oven temperature = 50ºC

b. Ramp = 20ºC/min

c. Inlet temperature = 230ºC

d. Detector temperature = 250ºC

e. H_2 flow = 40 mL/min

f. He flow = 179 mL/min

g. Air flow = 400 mL/min

7. Start the GC on the computer by selecting the method with the setting conditions above mentioned. Set and load the sequence of samples to run. Once the conditions are reached and the green start signal is on the screen, start running the sequence. Details about operation, setting sequence, and calibration are in the Agilent 6890 instrument manual.

8. Periodically check to ensure that the equipment is working properly.

9. After the sequence is done, switch the GC to standby status or add the standby status as an extra line to the GC sequence so that it will automatically go to standby once it done and turn off air and hydrogen cylinder connection to GC.

APPENDIX G

VOLATILE SOLIDS ANALYSIS

Procedure for Product Liquid

When approximately 900 mL of product liquid have been collected, take the collection bottle out of the freezer and leave the bottle to be thawed overnight. Sometimes, there is a small amount of solid particles in the collected product liquid that were inadvertently washed into the liquid collection bottle. To ensure an accurate measure, this amount of solids also needs to be analyzed for VS, so Steps 10–16 are needed.

1. Record the weight of the full collection (without cap).

2. Centrifuge the liquid collection bottle to separate any solids that might be in the liquid. Use the centrifuge for 20 min at 3500 rpm. When finished, decant all the supernatant liquid into a large clean empty container, being careful not to lose any solids from the bottles.

3. Record the weight of an empty 500-mL Erlenmeyer flask.

4. Add approximately 3 g $Ca(OH)_2$ to the empty container and record weight.

5. Add approximately 100 g of supernatant liquid to the container and record the weight. Mix well. Throw away the rest of the liquid.

6. Record the label and weight of a clean, dry, 150-mL crucible (Crucible A).

7. Pour, while mixing, approximately 70 g of the lime and liquid product mix into Crucible A. Record weight of the Crucible A + liquid mix.

8. Dry the crucible at 105°C for 2 days in the drying oven. Place the crucible in a vacuum dessicator and allow it to cool to room temperature before weighing. Record the weight of the crucible.

9. Ash the crucible at 550°C for at least 2 h. Remove the crucible from the ashing oven and place it in a vacuum dessicator and allow it to cool to room temperature. Record the ash weight of the crucible.

10. Record the weight of the collection bottle after pouring off all the liquid.

11. Record the label and weight of a clean, dry, 150-mL crucible (Crucible B).

12. Add approximately 3 g of $Ca(OH)_2$ to Crucible B and record weight.

13. Mix the remaining content of the liquid collection bottle, and pour carefully approximately 70 g into Crucible B. Mix well the lime and solids, and record the weight of the crucible.

14. Dry the crucible at 105°C as in Step 8.

15. Ash the crucible at 550°C as in Step 9.

16. Wash, dry and record the weight of the empty liquid collection bottle (without cap)

The amount of VS in the supernatant liquid is calculated as:

$$VS_{dissolved}(g\ VS) = \frac{(W8-W9)}{\left(\frac{W7-W6}{W5-W3}\right)\times\left(\frac{W5-W4}{W1-W10}\right)}$$

$$VS_{dissolved}(g\ VS/(g.d)) = \frac{\frac{(W8-W9)}{\left(\frac{W7-W6}{W5-W3}\right)\times\left(\frac{W5-W4}{W1-W10}\right)}}{\text{collected time period}}$$

The amount of VS in the solid residue present in the liquid is calculated as:

$$VS_{\text{solid residue}}(\text{g VS}) = \frac{(W14 - W15)}{\left(\frac{W13 - W15}{W10 - W16}\right)}$$

In all the formulas, W*i* is the weight recorded in the i_{th} step.

Procedure for Solid

1. Record the weight of the full collection bottle (without cap).

2. Empty the solids into a clean empty container, and mix very well. Be careful not to lose any solids from the bottle.

3. Record the label and weight of a clean, dry, 150-mL crucible.

4. Remove a representative sample of approximately 10 g of solid product into the crucible, and record the weight of the crucible

5. Dry the crucible at 105°C for 2 days in the drying oven. Place the crucible in a vacuum dessicator and allow cooling to room temperature before weighing. Record the dry weight of the crucible.

6. Ash the crucible at 550°C for at least 2 h. Remove quickly the crucible from the ashing oven and place it in a vacuum dessicator and allow cooling to room temperature. Record the ash weight of the crucible.

7. Record the weight of the empty liquid collection bottle (without cap).

The amount of VS in the solid is calculated as:

$$VS_{\text{Solid}} = \frac{(W5 - W6)}{\left(\frac{W4 - W3}{W1 - W7}\right)}$$

The amount of VS in one gram of collected solids is calculated as:

$$VS_{g\ solid}(\text{g VS/g solids}) = \frac{(W5 - W6)}{(W4 - W3)}$$

Again, in all the formulas, W*i* represents the weight recorded in the i_{th} step.

APPENDIX H

CPDM MATHEMATICA PROGRAM

This appendix contains the CPDM *Mathematica* program used to obtain the predicted product concentration and substrate conversion at various VSLR and LRT. The program results are acid concentration (g acetic acid equivalents/L) and conversion in each fermentor. To determine the total carboxylic acid concentration, the acetic acid equivalent concentration is multiplied by ϕ (ratio of g total acid/g aceq). The constant values for the system-specific parameters are denoted with "**". VSLR and LRT are the independent variables for constructing the CPDM "map."

```
k=3.5;
While[k<3.51,
l=1;
While[l<1.01,

holdup = 4,27;                  **ratio of liquid to solid in wet cake (g liquid/g VS wet
                                  cake) Amount of liquid dragged by the solids. This
                                  number is obtained from the moisture content of the
                                  solids at the  outlet of the fermentor. **
moist =0.08;                     **weight ratio of liquid in biomass feed
                                  (g liquid/g VS in feed)**
so = 0.65;                       **selectivity, σ (g Aceq/g VS digested)**
ratio = 0.90;                    **ratio of g total acid to g Aceq**
stages = 4;
loading =10;                      **VSLR**
tauloverall = 25;                 **LRT**
vol = {.210,.210,.210,.210};      ** individual liquid volume in fermentors**
totvol = Sum[vol[[i]],{i,1,stages}];
liquidfeed = totvol/tauloverall;
nnotreal = {300,300,300,300};     **VS concentration (g VS/L)**
solidfeed = loading totvol;
Convrsn = {.1,.2,.3,.4};
nnot = nnotreal/(1-Convrsn);
taus = nnot*vol/solidfeed;
L = Table[0.1, {i, 1, stages+1}];
taul = Table[tauloverall/stages, {i, 1, stages}];
```

```
fit = {e ->0.26, f->2.66, g->3.43, h ->0.461};        **CPDM parameters**

** The following codes do not require modification from the user**

rmodel[x_, acd_]:= e (1-x)^f/(1+g (acd*ratio)^h)/.fit;     **Eq. (4-12)**
rmodel[x, acd];
slp = D[rmodel[x,ac], x];
drmodel[xx_, aac_]:= slp /. {x->xx, ac ->aac};
drmodel[x, ac];
acid =\[InvisibleSpace]{20,10,10,5};
ans=Table[1, {i,1,stages}];
tauloverallnew=20;
taulnew = Table[1000, {i, 1, stages}];
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nhatzero = Table[100, {i, 1, stages}];
done = 0;
liqtoler = 0.05;
acidtoler = 0.02;
nnottoler = 1;
done = 0;
acidold = Table[1.0, {i, 1, stages}];
creation = Table[1, {i, 1, stages}];
destruction = Table[1, {i, 1, stages}];
While[done < 0.50, {taulnew = Table[10000, {j, 1, stages}];
While[Abs[tauloverall-tauloverallnew] > 0.01,
liquidfeed = liquidfeed (1 + (tauloverallnew-tauloverall)/tauloverall * .5);
L[[5]] = liquidfeed;
L[[4]] = L[[5]] + solidfeed/1000 holdup (Convrsn[[4]]-Convrsn[[3]]);
L[[3]] = L[[4]] + solidfeed/1000 holdup (Convrsn[[3]]-Convrsn[[2]]);
L[[2]] = L[[3]] + solidfeed/1000 holdup (Convrsn[[2]]-Convrsn[[1]]);
L[[1]] = moist solidfeed/1000 + L[[2]] - solidfeed/1000 holdup (1.0-Convrsn[[1]]);
tauloverallnew = totvol/L[[1]]; ];
taul = Table[vol[[j]]/L[[j]], {j, 1, stages}];
scale = Table[1, {j, 1, stages}];
nnot = nnotreal/(1-Convrsn);
taus = nnot*vol/solidfeed;
Print[nnot];
i=1;
While[Abs[taulnew[[i]] - taul[[i]]] > liqtoler,
{ans[[i]] = NDSolve[{nhat[0] == 10,
nhat'[x] == -nhat[x] ( drmodel[x, acid[[i]] ] + so/taus[[i]] )/(rmodel[x, acid[[i]] ])},
nhat[x], {x, 0, 0.99}];
factr1 = nnot[[i]]/NIntegrate[ (nhat[x] /. ans[[i]] )[[1]], {x, 0, 0.99}];
robs = NIntegrate[factr1 (nhat[x] /. ans[[i]] )[[1]] (rmodel[x, acid[[i]] ] ), {x, 0, 0.99}];
```

```
Convrsn[[i]] = NIntegrate[x (nhat[x]/.ans[[1]][[1]]), {x, 0, 0.99}]/nnot[[1]] factr1;
taulnew[[i]] = (L[[i]] acid[[i]] + solidfeed/1000 (1 - Convrsn[[i]]) holdup acid[[i]] -
L[[i+1]]*
acid[[i+1]])/(L[[i]] robs) ;
acid[[i]] = acid[[i]] + (taul[[i]] robs -(L[[i]] acid[[i]] + solidfeed/1000 (1 - Convrsn[[i]])
holdup*
acid[[i]] - L[[i+1]]* acid[[i+1]])/L[[i]] ) 0.4;}];
Print["acid", i, "=", acid[[i]], " taulnew", i, "=", taulnew[[i]] , "robs =",robs];
i=2;
nnottoler = nnot[[i]]/500;
While[Abs[taulnew[[i]] - taul[[i]]] > liqtoler, {ndone = 0;While[ndone < 0.50,
{ans[[i]] = NDSolve[{nhat[0] == nhatzero[[i]], nhat'[x] == -nhat[x] ( drmodel[x,
acid[[i]]
] + so/taus[[i]] )/(rmodel[x, acid[[i]] ]) +(nhat[x]/. ans[[i-1]][[1]] ) nnot[[i]]/nnot[[i-1]]
factr1 (so/(taus[[i]] rmodel[x, acid[[i]] ] ) ) },nhat[x], {x, 0, 0.99}];
nhattot = NIntegrate[(nhat[x]/. ans[[i]])[[1]], {x, 0, 0.99}];
Print["nhatzero=", nhatzero[[i]], " nhattot=", nhattot, "nnot[[i]]=",nnot[[i]] ];
ndone = If[Abs[nhattot - nnot[[i]] ] < nnottoler, 1, 0];
nhatzero[[i]] = If[nhatzero[[i]] + (nnot[[i]] - nhattot) 1.0 > 0,
nhatzero[[i]] + (nnot[[i]] - nhattot)/nnot[[i]] 50 ,
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nhatzero[[i]] + (nnot[[i]] - nhattot)/nnot[[i]] 50 ] } ];
Convrsn[[i]] = (NIntegrate[x (nhat[x]/.ans[[i]][[1]]), {x, 0, 0.99}])/nnot[[i]];
robs = solidfeed so/vol[[i]] (Convrsn[[i]] - Convrsn[[i-1]]);
taulnew[[i]] = (L[[i]] acid[[i]] + solidfeed/1000 (1 - Convrsn[[i]] ) holdup acid[[i]]
- L[[i+1]] acid[[i+1]] -solidfeed/1000 (1 - Convrsn[[i-1]] ) holdup acid[[i-1]])/
(L[[i]] robs);
acid[[i]] = acid[[i]] + (taul[[i]] robs - (L[[i]] acid[[i]] + solidfeed/1000
(1 - Convrsn[[i]] ) holdup acid[[i]] - L[[i+1]] acid[[i+1]] -
solidfeed/1000 (1 - Convrsn[[i-1]] ) holdup acid[[i-1]])/L[[i]]) 0.5;}];
Print["acid", i, "=", acid[[i]], " taulnew", i, "=", taulnew[[i]] , "robs =",robs];
i=3;
nnottoler = nnot[[i]]/500;
While[Abs[taulnew[[i]] - taul[[i]]] > liqtoler,
{ndone = 0;
While[ndone < 0.50,
{ans[[i]] = NDSolve[{nhat[0] == nhatzero[[i]],
nhat'[x] == -nhat[x] ( drmodel[x, acid[[i]] ] + so/taus[[i]] )/(rmodel[x, acid[[i]] ]) +
(nhat[x]/. ans[[i-1]][[1]] ) nnot[[i]]/nnot[[i-1]] (so/(taus[[i]] rmodel[x, acid[[i]] ] ) ) },
nhat[x], {x, 0, 0.99}];
nhattot = NIntegrate[(nhat[x]/. ans[[i]])[[1]], {x, 0, 0.99}];
Print["nhatzero=", nhatzero[[i]], " nhattot=", nhattot, "nnot[[i]]=",nnot[[i]] ];
ndone = If[Abs[nhattot - nnot[[i]] ] < nnottoler, 1, 0];
nhatzero[[i]] = If[nhatzero[[i]] + (nnot[[i]] - nhattot) 1.0 > 0,
```

```
nhatzero[[i]] + (nnot[[i]] - nhattot)/nnot[[i]] 25 ,
nhatzero[[i]] + (nnot[[i]] - nhattot)/nnot[[i]] 25 ] } ];
Convrsn[[i]] = (NIntegrate[x (nhat[x]/.ans[[i]][[1]]), {x, 0, 0.99}])/nnot[[i]];
robs = solidfeed so/vol[[i]] (Convrsn[[i]] - Convrsn[[i-1]]);
Convrsn[[i]] = (NIntegrate[x (nhat[x]/.ans[[i]][[1]]), {x, 0, 0.99}])/nnot[[i]];
taulnew[[i]] = (L[[i]] acid[[i]] + solidfeed/1000 (1 - Convrsn[[i]] ) holdup acid[[i]]
- L[[i+1]] acid[[i+1]] -solidfeed/1000 (1 - Convrsn[[i-1]] ) holdup acid[[i-1]])/
(L[[i]] robs);
acid[[i]] = acid[[i]] + (taul[[i]] robs - (L[[i]] acid[[i]] + solidfeed/1000
(1 - Convrsn[[i]] ) holdup acid[[i]] - L[[i+1]] acid[[i+1]] -
solidfeed/1000 (1 - Convrsn[[i-1]] ) holdup acid[[i-1]])/L[[i]]) 0.5;} ];
Print[" acid", i, "=", acid[[i]], " taulnew", i, "=", taulnew[[i]] , "robs=",robs];
i = 4;
nnottoler = nnot[[i]]/500;
scale[[4]]=0.5;
While[Abs[taulnew[[i]] - taul[[i]]] > liqtoler,
{ndone = 0;
While[ndone < 0.50,
{ans[[i]] = NDSolve[{nhat[0] == nhatzero[[i]],
nhat'[x] == -nhat[x] ( drmodel[x, acid[[i]] ] + so/taus[[i]] )/(rmodel[x, acid[[i]] ]) +
(nhat[x]/. ans[[i-1]][[1]] ) nnot[[i]]/nnot[[i-1]] (so/(taus[[i]] rmodel[x, acid[[i]] ] ) ) },
nhat[x], {x, 0, 0.99}];
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nhattot = NIntegrate[(nhat[x]/. ans[[i]])[[1]], {x, 0, 0.99}];
Print["nhatzero=", nhatzero[[i]], " nhattot=", nhattot, "nnot[[i]]=",nnot[[i]] ];
ndone = If[Abs[nhattot - nnot[[i]] ] < nnottoler, 1, 0];
nhatzero[[i]] = If[nhatzero[[i]] + (nnot[[i]] - nhattot) 1.0 > 0,
nhatzero[[i]] + (nnot[[i]] - nhattot)/nnot[[i]] 25 ,
nhatzero[[i]] + (nnot[[i]] - nhattot)/nnot[[i]] 25 ] } ];
Convrsn[[i]] = (NIntegrate[x (nhat[x]/.ans[[i]][[1]]), {x, 0, 0.99}])/nnot[[i]];
robs = solidfeed so/vol[[i]] (Convrsn[[i]] - Convrsn[[i-1]]);
taulnew[[i]] = (L[[i]] acid[[i]] + solidfeed/1000 (1 - Convrsn[[i]] ) holdup acid[[i]]
-solidfeed/1000 (1 - Convrsn[[i-1]] ) holdup acid[[i-1]])/(L[[i]] robs);
acid[[i]] = acid[[i]] + (taul[[i]] robs - (L[[i]] acid[[i]] + solidfeed/1000
(1 - Convrsn[[i]] ) holdup acid[[i]] -
solidfeed/1000 (1 - Convrsn[[i-1]] ) holdup acid[[i-1]])/L[[i]]) 0.5;} ];
Print[" acid", i, "=", acid[[i]], " taulnew", i, "=", taulnew[[i]] , "robs =",robs];
Convrsn=Flatten[{NIntegrate[x (nhat[x]/.ans[[1]][[1]]), {x, 0, 0.99}]/nnot[[1]] factr1,
Table[(NIntegrate[x (nhat[x]/.ans[[i]][[1]]), {x, 0, 0.99}])/nnot[[i]],
i,2,stages}]}];Print["conversion in each stage (from nhat)", Convrsn];
done = If[Max[Abs[(acidold-acid)] ] < acidtoler, 1, 0]; acidold = acid}]
Print[L[[1]]];
Print[L[[2]]];
Print[L[[3]]];
```

```
Print[L[[4]]];
Print[L[[5]]];
creation[[1]] = L[[1]] acid[[1]] + solidfeed/1000 (1 - Convrsn[[1]]) holdup acid[[2]] -
L[[2]] acid[[2]] ;
creation[[2]] = L[[2]] acid[[2]] + solidfeed/1000 (1 - Convrsn[[2]]) holdup acid[[3]] -
L[[3]] acid[[3]] -
solidfeed/1000 (1 - Convrsn[[1]]) holdup acid[[2]];
creation[[3]] = L[[3]] acid[[3]] + solidfeed/1000 (1 - Convrsn[[3]]) holdup acid[[4]] -
L[[4]] acid[[4]] -
solidfeed/1000 (1 - Convrsn[[2]]) holdup acid[[3]];
creation[[4]] = L[[4]] acid[[4]] - solidfeed/1000 (1 - Convrsn[[3]]) holdup acid[[4]];
destruction[[1]] = solidfeed/1000 (Convrsn[[1]] - 0);
destruction[[2]] = solidfeed/1000 (Convrsn[[2]] - Convrsn[[1]]);
destruction[[3]] = solidfeed/1000 (Convrsn[[3]] - Convrsn[[2]]);
destruction[[4]] = solidfeed/1000 (Convrsn[[4]] - Convrsn[[3]]);
Print["Selectivity = ",creation/destruction];
Print["Creation = ", creation];
Print["destruction = ",destruction];
selec = L[[1]] acid[[1]]/(solidfeed Convrsn[[4]]);
Print["selectivity = ",selec];
Print["k = ",k," l = ",l];
Print["loading = ", loading];
Print["tauloverall ", tauloverall];
Print["taus ", Sum[taus[[i]], {i, 1, stages}]];
Print["acid levels ",acid];
l = l + 0.5;];
k = k + 0.5;];
```

APPENDIX I

MATLAB CODE FOR CPDM MAP

The following code was developed by Fu (2007). It was slightly modified and used in this research. Data from an Excel spreadsheet was imported into Matlab. Individual variables for VSLR, LRT, CONVERSION, and ACID were then created in Matlab with the data from the imported spreadsheet. Once that was done, the code below was pasted and this created the CPDM maps. For superimposing one figure on another, which is done in all the comparisons, one figure is initially created and saved but the figure window should not be closed. Then another set of data is imported and the code is applied again. This will create two maps on the same figure. Manual formatting of the axes and figure can be done using the Matlab inspector.

```
mapdata=[VSLR,LRT,CONVERSION,ACID];
VSLR_sorted=sortrows(mapdata,1); %sort
LRT_sorted=sortrows(mapdata,2); %sort
[map_num,map_1]=size(mapdata);
VSLR_sort = sort(mapdata(:,1));
uniqueM = [diff(VSLR_sort);1] > 0;
%count = [VSLR_sort(uniqueM); diff(find([1;uniqueM]))]
VSLR_sort1 = VSLR_sort(uniqueM);
VSLR_number = diff(find([1;uniqueM]));
LRT_sort = sort(mapdata(:,2));
uniqueM = [diff(LRT_sort);1] > 0;
%count = [sortM(uniqueM) diff(find([1;uniqueM]))]
LRT_sort1 = LRT_sort(uniqueM); %Unique LRT
LRT_number = diff(find([1;uniqueM]));
temp1=zeros(length(VSLR_sort1)+1,1);
for j1=1:length(VSLR_sort1)
temp1(j1+1)=temp1(j1)+VSLR_number(j1);
mapdata_1=VSLR_sorted(temp1(j1)+1:temp1(j1+1),:) ;
%for VSLR(j1)
F = @(x)interp1(mapdata_1(:,3),mapdata_1(:,4),x,'spline');
hold on;
plot(mapdata_1(:,3),F(mapdata_1(:,3)),'k');
if j1==1
for j3=1:length(mapdata_1(:,3))
text(mapdata_1(j3,3),mapdata_1(j3,4), [' ', num2str(mapdata_1(j3,2))] , 'HorizontalAlignment','left');
```

```
end
end
% text(a(3),b(3), ' LRT (day)' , 'HorizontalAlignment','left');
end
temp1=zeros(length(LRT_sort1)+1,1);
%temp1(1)=LRT_number(1);
for j1=1:length(LRT_sort1)
temp1(j1+1)=temp1(j1)+LRT_number(j1);
mapdata_2=LRT_sorted(temp1(j1)+1:temp1(j1+1),:) ;
%for LRT(j1)
F2 = @(x)interp1(mapdata_2(:,3),mapdata_2(:,4),x,'spline');
hold on;
plot(mapdata_2(:,3),F2(mapdata_2(:,3)),'k');
if j1==1
for j3=1:length(mapdata_2(:,3))
text(mapdata_2(j3,3),mapdata_2(j3,4), [' ', num2str(mapdata_2(j3,1))] ,
'HorizontalAlignment','right');
end
end
% text(a(3),b(3), ' LRT (day)' , 'HorizontalAlignment','left');
end
hold off;
xlabel (' Fermentation Conversion');
ylabel ('Total carboxylic acid concentration (g/L)');
axis([0 1 0 80]);
```

APPENDIX J

CARBOXYLIC ACID PRODUCTION DATA AND MASS BALANCE CALCULATIONS FOR SUGARCANE TRASH COUNTERCURRENT FERMENTATIONS

Table J-1. Carboxylic acid production in F1 for Train A (Long-term air-lime pretreated bagasse, ammonium bicarbonate buffer, and 55°C)

Acid Production for Train A							
Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)
2	16.880	0.268	1.828	0.204	0.000	0.000	19.180
4	18.457	0.297	1.465	0.154	0.000	0.000	20.373
6	18.551	0.293	1.424	0.145	0.000	0.000	20.413
8	19.154	0.287	1.666	0.162	0.000	0.000	21.268
10	15.805	0.335	5.008	0.153	0.000	0.000	21.301
12	17.917	0.444	4.562	0.168	0.000	0.000	23.092
14	17.876	0.465	4.601	0.122	0.000	0.000	23.063
16	18.393	0.510	4.379	0.120	0.000	0.000	23.403
18	21.886	0.724	4.447	0.147	0.000	0.000	27.204
20	24.414	0.824	4.573	0.158	0.000	0.021	29.989
22	25.003	0.790	4.314	0.127	0.000	0.000	30.234
24	25.077	0.778	4.185	0.129	0.000	0.000	30.170
26	17.637	0.556	2.914	0.109	0.000	0.000	21.216
28	25.379	0.922	4.177	0.175	0.000	0.000	30.653
30	22.148	0.830	3.735	0.162	0.000	0.000	26.875
32	23.507	0.850	4.074	0.156	0.000	0.000	28.587
34	23.744	0.837	3.848	0.159	0.000	0.000	28.588
36	25.273	0.849	3.631	0.160	0.000	0.000	29.913
38	28.379	0.956	3.249	0.175	0.000	0.000	32.759
40	28.180	1.023	3.488	0.155	0.000	0.000	32.846
42	26.864	0.982	3.619	0.132	0.000	0.048	31.645
44	26.152	0.976	3.810	0.098	0.000	0.051	31.087
46	28.549	1.081	4.527	0.073	0.000	0.045	34.275
48	24.316	0.913	3.340	0.056	0.000	0.046	28.672
50	33.811	1.239	4.304	0.056	0.000	0.056	39.466
52	34.345	1.252	4.389	0.000	0.000	0.054	40.040
54	36.487	1.275	4.882	0.057	0.000	0.000	42.701
56	34.379	1.239	5.300	0.055	0.000	0.000	40.972
58	35.404	1.331	6.235	0.067	0.000	0.000	43.038
60	37.173	1.421	7.163	0.081	0.000	0.000	45.837
62	35.080	1.346	7.298	0.073	0.000	0.000	43.797
64	34.243	1.311	7.485	0.064	0.000	0.000	43.102
66	32.745	1.269	7.135	0.059	0.000	0.000	41.208

Table J-1. (Continued)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)
68	30.260	1.240	6.222	0.053	0.000	0.000	37.775
70	29.172	1.218	5.671	0.054	0.000	0.000	36.116
72	27.564	1.251	6.355	0.056	0.000	0.000	35.226
74	27.592	1.296	7.400	0.063	0.000	0.000	36.350
76	26.614	1.205	5.923	0.066	0.000	0.000	33.809
78	27.284	1.261	7.517	0.071	0.000	0.000	36.134
80	26.702	1.226	7.314	0.071	0.000	0.000	35.312
82	25.665	1.193	7.042	0.080	0.000	0.000	33.981
84	25.966	1.287	7.091	0.099	0.000	0.000	34.443
86	24.079	1.440	6.225	0.103	0.053	0.000	31.900
88	22.038	1.416	5.481	0.096	0.000	0.000	29.032
90	21.554	1.425	5.121	0.089	0.000	0.000	28.189
92	22.294	1.423	5.280	0.087	0.000	0.000	29.084
94	21.213	1.311	5.072	0.077	0.064	0.000	27.737
96	22.515	1.260	4.861	0.070	0.000	0.000	28.706
98	21.739	1.146	4.584	0.000	0.076	0.000	27.544
100	21.851	1.100	4.527	0.000	0.000	0.000	27.478
102	21.951	1.155	4.893	0.000	0.000	0.000	27.999
104	21.401	1.160	4.760	0.000	0.000	0.000	27.320
106	20.736	1.241	4.919	0.000	0.000	0.000	26.896
108	19.407	1.190	4.538	0.000	0.060	0.000	25.195
110	20.882	1.298	4.737	0.074	0.072	0.000	27.063
112	19.999	1.262	4.602	0.079	0.068	0.000	26.009
114	20.231	1.310	4.797	0.075	0.088	0.000	26.501
116	20.213	1.312	4.839	0.090	0.077	0.000	26.531
118	17.821	1.147	4.058	0.080	0.083	0.000	23.189
120	20.563	1.336	4.754	0.096	0.095	0.000	26.844
122	20.366	1.307	4.695	0.098	0.085	0.000	26.552
124	20.548	1.334	4.559	0.101	0.099	0.000	26.640
126	20.642	1.339	4.332	0.099	0.082	0.000	26.493
128	21.448	1.339	4.109	0.094	0.080	0.000	27.070
130	21.504	1.298	3.836	0.079	0.102	0.000	26.819
132	21.690	1.295	3.964	0.086	0.092	0.000	27.127
134	20.287	1.205	4.040	0.092	0.082	0.000	25.705
136	19.529	1.153	3.887	0.092	0.077	0.000	24.738
138	21.309	1.245	4.089	0.100	0.075	0.000	26.818
140	20.937	1.213	3.906	0.093	0.079	0.000	26.229
142	19.781	1.117	3.579	0.089	0.079	0.000	24.646
144	19.677	1.118	3.402	0.083	0.075	0.000	24.354
146	20.103	1.151	3.497	0.070	0.078	0.000	24.900
148	20.481	1.193	3.673	0.070	0.086	0.000	25.503
150	21.390	1.235	3.886	0.075	0.084	0.000	26.671
152	19.865	1.156	3.692	0.073	0.078	0.000	24.863
154	20.281	1.148	3.583	0.070	0.067	0.000	25.151
156	20.961	1.195	3.752	0.066	0.082	0.000	26.056

Table J-2. Carboxylic acid production in F1 for Train B (Long-term air-lime pretreated bagasse, ammonium bicarbonate buffer, and 55°C)

Acid Production for Train B							
Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)
2	16.573	0.224	1.063	0.150	0.000	0.000	18.010
4	20.215	0.296	1.821	0.164	0.000	0.000	22.496
6	21.826	0.274	2.136	0.169	0.000	0.000	24.404
8	21.980	0.292	2.392	0.186	0.000	0.000	24.851
10	8.582	0.213	6.423	0.085	0.000	0.000	15.303
12	17.943	0.412	7.050	0.118	0.000	0.000	25.524
14	18.614	0.422	6.989	0.093	0.000	0.000	26.118
16	20.052	0.464	5.728	0.084	0.000	0.000	26.328
18	24.993	0.615	5.586	0.124	0.000	0.000	31.318
20	26.930	0.645	5.839	0.160	0.000	0.000	33.574
22	29.511	0.688	5.482	0.169	0.000	0.000	35.850
24	28.081	0.670	4.885	0.156	0.000	0.000	33.793
26	28.257	0.722	5.191	0.156	0.000	0.000	34.325
28	27.965	0.709	4.917	0.146	0.000	0.000	33.736
30	30.801	0.797	4.496	0.169	0.000	0.000	36.264
32	28.191	0.953	3.273	0.130	0.000	0.000	32.547
34	29.109	1.100	3.210	0.130	0.000	0.000	33.549
36	29.292	1.126	3.664	0.119	0.000	0.000	34.201
38	30.286	1.104	3.799	0.127	0.000	0.000	35.315
40	35.141	1.265	4.516	0.138	0.000	0.000	41.061
42	30.519	1.081	3.461	0.103	0.000	0.000	35.164
44	30.594	1.004	3.519	0.070	0.000	0.000	35.187
46	32.101	0.946	3.238	0.000	0.000	0.000	36.285
48	37.180	1.137	3.616	0.000	0.000	0.000	41.933
50	34.397	0.983	3.789	0.000	0.000	0.058	39.227
52	38.132	1.080	4.158	0.000	0.000	0.000	43.371
54	34.202	1.015	3.860	0.059	0.000	0.000	39.136
56	35.308	1.135	4.685	0.077	0.000	0.000	41.206
58	33.678	1.075	4.855	0.053	0.000	0.000	39.660
60	33.047	1.053	5.148	0.077	0.058	0.000	39.383
62	33.993	1.080	5.600	0.067	0.049	0.000	40.789
64	33.823	1.077	5.481	0.068	0.000	0.000	40.448
66	31.652	1.038	5.352	0.064	0.000	0.000	38.106
68	28.877	1.000	5.903	0.055	0.000	0.000	35.835
70	24.646	0.913	5.243	0.000	0.000	0.000	30.803
72	27.968	1.123	6.458	0.000	0.000	0.000	35.549
74	27.632	1.174	6.631	0.000	0.000	0.000	35.437
76	27.429	1.229	6.653	0.000	0.000	0.000	35.310
78	26.875	1.244	6.871	0.000	0.000	0.000	34.990
80	26.030	1.235	6.906	0.000	0.000	0.000	34.171
82	24.652	1.204	7.245	0.055	0.000	0.000	33.156
84	24.132	1.171	7.035	0.061	0.000	0.000	32.398

Table J-2. (Continued)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)
86	23.923	1.238	6.427	0.058	0.000	0.000	31.647
88	21.784	1.195	6.041	0.000	0.000	0.000	29.020
90	22.373	1.250	5.922	0.000	0.000	0.000	29.545
92	22.274	1.244	5.957	0.058	0.000	0.000	29.534
94	21.945	1.227	5.831	0.000	0.000	0.000	29.003
96	24.176	1.193	5.550	0.000	0.000	0.000	30.919
98	21.057	1.010	5.631	0.000	0.000	0.000	27.698
100	22.971	1.120	6.089	0.000	0.000	0.000	30.180
102	22.331	1.106	5.928	0.000	0.000	0.000	29.365
104	22.884	1.181	5.974	0.000	0.000	0.000	30.039
106	21.860	1.226	5.888	0.000	0.000	0.000	28.974
108	21.992	1.304	5.172	0.000	0.064	0.000	28.532
110	22.233	1.361	5.515	0.000	0.065	0.000	29.175
112	22.024	1.392	5.873	0.000	0.066	0.000	29.355
114	21.040	1.351	5.800	0.073	0.068	0.000	28.332
116	20.196	1.309	5.420	0.076	0.079	0.000	27.079
118	20.127	1.324	5.241	0.074	0.085	0.000	26.851
120	20.142	1.346	6.071	0.076	0.084	0.000	27.719
122	18.428	1.229	5.466	0.069	0.062	0.000	25.254
124	19.102	1.386	5.921	0.076	0.125	0.000	26.611
126	19.070	1.393	5.743	0.071	0.076	0.000	26.353
128	18.836	1.395	5.522	0.071	0.098	0.000	25.922
130	19.176	1.405	5.313	0.067	0.095	0.000	26.056
132	18.584	1.321	5.857	0.059	0.087	0.000	25.908
134	19.071	1.351	6.022	0.061	0.085	0.000	26.590
136	19.679	1.416	5.118	0.063	0.069	0.000	26.344
138	19.589	1.366	4.996	0.061	0.065	0.000	26.077
140	19.488	1.381	5.900	0.060	0.075	0.000	26.903
142	19.728	1.427	4.962	0.075	0.067	0.000	26.259
144	19.344	1.396	4.730	0.073	0.066	0.000	25.609
146	20.826	1.449	4.834	0.084	0.076	0.000	27.269
148	20.792	1.398	4.736	0.084	0.078	0.000	27.089
150	20.818	1.346	4.525	0.090	0.070	0.000	26.849
152	21.103	1.347	4.668	0.098	0.077	0.000	27.292
154	18.832	1.181	4.280	0.089	0.072	0.000	24.453
156	20.922	1.314	4.660	0.098	0.080	0.000	27.074

Table J-3. Carboxylic acid production in F1 for Train C (Long-term air-lime pretreated bagasse, ammonium bicarbonate buffer, and 55°C)

Acid Production for Train C							
Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)
2	17.381	0.227	1.276	0.170	0.000	0.000	19.054
4	19.023	0.297	1.862	0.151	0.000	0.000	21.333
6	19.968	0.328	2.955	0.210	0.000	0.000	23.461
8	20.946	0.361	2.842	0.205	0.000	0.000	24.355
10	22.707	0.387	3.714	0.199	0.000	0.000	27.008
12	23.838	0.434	3.660	0.189	0.000	0.000	28.121
14	26.306	0.537	3.421	0.163	0.000	0.000	30.427
16	24.263	0.584	3.448	0.119	0.000	0.000	28.414
18	21.064	0.565	3.459	0.100	0.000	0.042	25.231
20	23.368	0.663	4.442	0.128	0.000	0.000	28.602
22	20.573	0.649	4.190	0.112	0.000	0.045	25.570
24	22.156	0.794	4.911	0.128	0.000	0.000	27.988
26	23.821	1.027	5.195	0.156	0.000	0.047	30.245
28	25.473	1.209	5.288	0.165	0.000	0.048	32.184
30	29.104	2.568	5.024	0.203	0.000	0.000	36.899
32	30.518	3.332	5.548	0.199	0.000	0.000	39.598
34	28.315	2.989	4.311	0.164	0.000	0.000	35.779
36	27.615	2.289	4.449	0.130	0.000	0.000	34.483
38	25.616	1.912	4.031	0.108	0.000	0.000	31.666
40	26.992	1.748	4.495	0.102	0.000	0.000	33.337
42	31.825	1.831	4.918	0.118	0.000	0.000	38.692
44	27.379	1.456	4.717	0.097	0.000	0.000	33.649
46	27.432	1.362	4.654	0.087	0.000	0.000	33.536
48	29.318	1.370	4.623	0.081	0.000	0.000	35.391
50	29.695	1.330	5.326	0.069	0.000	0.000	36.418
52	32.481	1.396	4.947	0.078	0.000	0.000	38.901
54	33.993	1.429	5.989	0.079	0.000	0.000	41.490
56	33.941	1.455	6.479	0.102	0.000	0.000	41.977
58	31.923	1.390	6.884	0.100	0.000	0.000	40.297
60	32.299	1.447	6.429	0.146	0.000	0.000	40.321
62	31.594	1.453	6.876	0.108	0.000	0.000	40.031
64	31.001	1.437	6.807	0.117	0.000	0.000	39.363
66	33.663	1.569	7.833	0.124	0.000	0.000	43.189
68	28.540	1.356	6.673	0.104	0.043	0.000	36.716
70	27.905	1.332	6.532	0.083	0.000	0.000	35.852
72	27.046	1.364	6.606	0.076	0.000	0.000	35.092
74	28.101	1.409	6.731	0.074	0.000	0.000	36.315
76	26.871	1.362	6.726	0.070	0.000	0.000	35.028
78	26.098	1.342	6.656	0.061	0.000	0.000	34.158
80	25.361	1.342	6.910	0.064	0.000	0.000	33.678
82	25.325	1.349	6.722	0.068	0.000	0.000	33.465
84	25.124	1.320	6.435	0.065	0.000	0.000	32.944

Table J-3. (Continued)

Day	C2 (g/L)	C3 (g/L)	C4 (g/L)	C5 (g/L)	C6 (g/L)	C7 (g/L)	Total (g/L)
86	24.519	1.297	6.297	0.065	0.000	0.000	32.177
88	22.876	1.273	5.987	0.062	0.000	0.000	30.198
90	20.864	1.209	5.281	0.000	0.000	0.000	27.354
92	23.231	1.330	5.565	0.060	0.000	0.000	30.186
94	20.148	1.164	4.901	0.000	0.000	0.000	26.213
96	24.026	1.244	6.184	0.074	0.000	0.000	31.528
98	22.760	1.090	5.601	0.000	0.000	0.000	29.451
100	24.194	1.117	6.090	0.000	0.000	0.000	31.402
102	24.474	1.217	6.258	0.000	0.000	0.000	31.949
104	24.760	1.282	6.416	0.000	0.000	0.000	32.458
106	24.204	1.401	6.617	0.069	0.083	0.000	32.374
108	22.565	1.342	5.872	0.000	0.000	0.000	29.779
110	24.644	1.527	6.290	0.000	0.062	0.000	32.523
112	25.006	1.595	6.431	0.065	0.066	0.000	33.163
114	22.402	1.449	5.956	0.073	0.073	0.000	29.954
116	22.251	1.507	6.108	0.088	0.077	0.000	30.031
118	20.172	1.359	5.501	0.087	0.081	0.000	27.200
120	21.547	1.517	5.849	0.099	0.085	0.000	29.096
122	21.076	1.511	5.733	0.104	0.092	0.000	28.516
124	21.952	1.564	5.881	0.110	0.099	0.000	29.605
126	21.851	1.534	5.621	0.106	0.080	0.000	29.192
128	20.562	1.403	5.345	0.090	0.072	0.000	27.472
130	21.831	1.493	5.583	0.090	0.098	0.000	29.095
132	20.883	1.402	5.104	0.072	0.086	0.000	27.547
134	22.514	1.518	5.443	0.072	0.088	0.000	29.634
136	22.044	1.482	5.537	0.072	0.080	0.000	29.216
138	22.909	1.593	6.046	0.085	0.073	0.000	30.707
140	22.896	1.592	6.007	0.073	0.070	0.000	30.638
142	21.594	1.472	5.780	0.084	0.068	0.000	28.997
144	22.709	1.552	5.924	0.075	0.069	0.000	30.330
146	21.893	1.427	5.587	0.079	0.080	0.000	29.066
148	21.973	1.445	5.754	0.087	0.077	0.000	29.336
150	21.802	1.352	5.619	0.089	0.074	0.000	28.936
152	21.636	1.297	5.471	0.093	0.071	0.000	28.567
154	21.833	1.287	5.311	0.090	0.072	0.000	28.593
156	22.595	1.317	5.370	0.087	0.073	0.000	29.441

Table J-4. Fermentation mass balance data for Train A

Mass Balance Closure for Fermentation Train A											
Day	Ave pH	VS in (g)	Liq out (mL)	Solid out (g)	Acid out (g)	VS solid out (g)	VS liq out (g)	VS residue (g)	Biotic CO_2 (g)	CH_4 (g)	Total Gas out (g)
96	6.79	5.88	84	7.6	2.41	3.46	1.24	0.09	0.256	0.021	1.193
98	6.91	5.88	80	6.5	2.20	1.88	1.48	0.07	0.189	0.018	1.125
100	6.76	5.88	84	17.5	2.35	2.98	1.55	0.07	0.275	0.000	1.793
102	6.73	5.88	87	10.0	2.24	1.95	1.61	0.07	0.324	0.009	1.687
104	6.8	5.88	84	7.1	2.29	2.37	1.55	0.07	0.328	0.000	1.42
106	6.76	5.88	86	11.5	2.31	2.58	1.59	0.07	0.211	0.000	1.341
108	6.71	5.88	76	17.5	2.23	2.98	1.40	0.07	0.323	0.004	1.419
110	6.78	5.88	82	11.5	2.22	1.88	1.51	0.07	0.345	0.005	1.584
112	6.71	5.88	82	20.6	1.92	3.51	1.51	0.07	0.269	0.000	1.345
114	6.8	5.88	74	2.9	2.25	2.46	1.37	0.06	0.169	0.004	1.335
116	6.73	5.88	78	12.2	2.07	2.08	1.44	0.07	0.312	0.001	1.529
118	6.69	5.88	73	11.7	2.18	1.84	0.99	0.06	0.353	0.000	1.527
120	6.75	5.88	76	13.0	2.25	2.04	1.03	0.06	0.392	0.000	1.572
122	6.67	5.88	75	19.9	1.99	3.13	1.02	0.06	0.342	0.002	1.516
124	6.66	5.88	74	10.2	1.97	1.60	1.00	0.06	0.328	0.017	1.583
126	6.77	5.88	84	0.0	2.23	0.00	1.14	0.07	0.303	0.001	1.706
128	6.79	5.88	82	5.8	2.22	0.91	1.11	0.06	0.417	0.017	1.842
130	6.9	5.88	74	12.7	1.98	2.00	1.00	0.06	0.360	0.022	1.644
132	6.82	5.88	77	28.0	2.09	4.40	1.04	0.06	0.171	0.002	1.501
134	6.65	5.88	70	30.2	2.00	4.74	0.95	0.05	0.323	0.023	1.48
136	6.81	5.88	76	0.2	2.07	0.03	1.03	0.06	0.287	0.012	1.479
138	6.86	5.88	72	9.4	1.93	1.48	0.98	0.06	0.253	0.005	1.482
140	6.84	5.88	68	24.8	1.78	3.90	0.92	0.05	0.144	0.004	1.278
142	6.71	5.88	84	9.2	2.07	1.22	1.00	0.10	0.013	0.007	1.326
144	6.92	5.88	84	3.0	2.05	0.40	1.00	0.10	0.164	0.007	1.463
146	6.85	5.88	86	5.0	2.14	0.66	1.03	0.11	0.054	0.007	1.415
148	6.92	5.88	76	9.6	1.94	1.27	0.91	0.09	0.117	0.007	1.352
150	6.74	5.88	84	11.8	2.24	1.56	1.00	0.10	0.345	0.015	1.7834
152	6.78	5.88	76	8.6	1.89	1.14	0.91	0.09	0.389	0.003	1.584
154	6.83	5.88	74	10.9	1.86	1.44	0.88	0.09	0.208	0.002	1.388
AVE	6.78	5.88	79	11.6	2.11	2.06	1.17	0.07	0.265	0.007	1.490
STD	0.076	0.00	5	7.4	0.16	1.19	0.25	0.02	0.101	0.007	0.172

VS digested (g)	3.74	VS undigested(g)	2.14
Water of Hydrolysis(g)	0.416	**Closure**	**91%**

Table J-5. Fermentation mass balance data for Train B

Mass Balance Closure for Fermentation Train B											
Day	Ave pH	VS in (g)	Liq out (mL)	Solid out (g)	Acid out (g)	VS solid out (g)	VS liq out (g)	VS residue (g)	Biotic CO_2 (g)	CH_4 (g)	Total Gas out (g)
96	6.84	7.06	83	14.6	2.57	3.25	1.13	0.09	0.287	0.000	1.851
98	6.86	7.06	74	23.9	2.39	3.76	1.02	0.06	0.425	0.000	1.649
100	6.76	7.06	80	14.2	2.41	3.13	1.10	0.07	0.434	0.000	1.404
102	6.82	7.06	72	11.6	2.35	1.82	1.35	0.06	0.315	0.005	1.632
104	6.82	7.06	81	21.8	2.34	3.43	1.12	0.07	0.365	0.001	1.462
106	6.72	7.06	84	16.5	2.43	2.60	1.16	0.07	0.318	0.001	1.177
108	6.78	7.06	78	16.6	2.23	2.61	1.27	0.07	0.441	0.000	1.709
110	6.77	7.06	84	10.1	2.45	2.35	1.16	0.07	0.342	0.005	1.411
112	6.74	7.06	84	15.5	2.41	3.18	1.87	0.07	0.312	0.015	1.369
114	6.83	7.06	76	19.0	2.38	2.99	1.05	0.07	0.356	0.007	1.231
116	6.82	7.06	84	16.1	2.27	3.01	1.16	0.07	0.313	0.002	1.341
118	6.7	7.06	76	17.8	2.04	2.59	0.87	0.06	0.282	0.009	1.167
120	6.75	7.06	68	20.6	2.38	3.00	0.78	0.05	0.293	0.000	1.377
122	6.76	7.06	60	21.5	2.15	3.13	0.69	0.05	0.265	0.001	1.14
124	6.75	7.06	72	17.5	1.92	2.55	0.82	0.06	0.175	0.013	1.254
126	6.75	7.06	70	8.4	1.99	1.22	1.35	0.06	0.309	0.020	1.341
128	6.83	7.06	78	24.9	2.02	3.63	0.89	0.06	0.157	0.010	1.301
130	6.87	7.06	80	17.3	2.08	2.52	1.87	0.06	0.264	0.017	1.439
132	6.77	7.06	68	30.0	2.05	4.37	1.46	0.05	0.441	0.002	1.407
134	6.81	7.06	58	37.0	1.54	5.39	0.66	0.05	0.483	0.016	1.349
136	6.79	7.06	85	4.9	2.24	0.71	0.97	0.07	0.128	0.004	1.422
138	6.83	7.06	84	14.8	2.19	2.16	1.09	0.07	0.364	0.007	1.633
140	6.81	7.06	78	24.0	2.10	3.50	1.10	0.06	0.277	0.008	1.447
142	6.66	7.06	80	10.7	2.10	1.61	1.03	0.11	0.280	0.014	1.486
144	6.74	7.06	69	13.3	2.10	2.00	0.89	0.09	0.340	0.006	1.538
146	6.85	7.06	72	0.0	2.10	0.00	0.93	0.10	0.370	0.013	1.513
148	7.03	7.06	74	19.8	2.25	2.97	0.95	0.10	0.280	0.014	1.444
150	6.82	7.06	80	31.8	2.15	4.78	1.03	0.11	0.320	0.024	1.558
152	6.81	7.06	70	27.7	2.14	4.16	0.90	0.09	0.040	0.022	1.14
154	6.8	7.06	71	10.1	2.10	1.52	0.91	0.10	0.240	0.013	1.227
AVE	6.80	7.06	76	17.7	2.20	2.80	1.08	0.07	0.307	0.008	1.414
STD	0.066	0.00	7	7.9	0.20	1.15	0.28	0.02	0.096	0.007	0.174

VS digested (g)	4.19	VS undigested(g)	2.87
Water of Hydrolysis(g)	0.465	**Closure**	**86%**

Table J-6. Fermentation mass balance data for Train C

Mass Balance Closure for Fermentation Train C											
Day	Ave pH	VS in (g)	Liq out (mL)	Solid out (g)	Acid out (g)	VS solid out (g)	VS liq out (g)	VS residue (g)	Biotic CO_2 (g)	CH_4 (g)	Total Gas out (g)
96	6.93	8.23	76	20.0	2.40	3.40	0.95	0.08	0.458	0.005	1.813
98	6.80	8.23	70	26.8	2.06	3.46	1.20	0.06	0.478	0.001	1.881
100	6.70	8.23	84	34.4	2.64	4.44	1.44	0.07	0.219	0.000	1.377
102	6.68	8.23	79	25.0	2.52	4.21	1.36	0.07	0.379	0.000	1.473
104	6.80	8.23	80	31.8	2.60	4.11	1.44	0.07	0.408	0.001	1.447
106	6.75	8.23	86	15.0	2.78	1.94	1.48	0.07	0.410	0.000	1.474
108	6.77	8.23	100	14.7	2.98	3.79	1.72	0.09	0.287	0.000	1.601
110	6.80	8.23	96	10.6	3.12	2.87	1.65	0.08	0.375	0.000	1.401
112	6.76	8.23	74	19.2	2.79	2.48	1.73	0.06	0.418	0.000	1.614
114	6.85	8.23	76	20.4	2.58	3.67	1.31	0.07	0.205	0.008	1.557
116	6.84	8.23	70	34.3	2.98	4.43	1.20	0.06	0.254	0.019	1.071
118	6.69	8.23	88	37.7	2.39	5.05	1.23	0.07	0.395	0.010	1.361
120	6.76	8.23	61	23.0	1.99	3.08	1.47	0.05	0.408	0.014	1.262
122	6.76	8.23	75	34.0	2.14	4.55	1.05	0.06	0.256	0.015	1.333
124	6.71	8.23	66	29.2	2.97	3.91	0.92	0.05	0.124	0.016	1.408
126	6.76	8.23	86	30.0	2.51	4.02	1.20	0.07	0.234	0.010	1.54
128	6.84	8.23	64	23.6	3.11	3.16	0.90	0.05	0.253	0.011	1.306
130	6.85	8.23	68	49.8	2.93	6.67	0.95	0.05	0.294	0.009	1.395
132	6.79	8.23	73	37.3	2.01	4.99	1.02	0.06	0.000	0.015	1.875
134	6.73	8.23	78	4.7	2.07	1.88	1.09	0.06	0.478	0.018	1.632
136	6.78	8.23	80	11.3	2.34	1.51	1.12	0.06	0.292	0.020	1.586
138	6.87	8.23	80	9.6	2.46	1.29	1.12	0.06	0.266	0.010	1.646
140	6.94	8.23	76	27.0	2.33	3.61	1.06	0.06	0.098	0.008	1.408
142	6.82	8.23	73	32.4	2.12	4.41	1.17	0.11	0.134	0.009	1.309
144	6.82	8.23	73	21.4	2.21	2.91	1.17	0.11	0.193	0.010	1.293
146	6.87	8.23	66	17.8	1.92	2.42	1.05	0.10	0.209	0.015	1.27
148	6.85	8.23	64	29.8	2.55	4.05	1.02	0.09	0.236	0.013	1.277
150	6.80	8.23	64	45.9	2.77	6.24	1.02	0.09	0.303	0.019	1.3222
152	6.85	8.23	68	20.3	2.79	2.76	1.09	0.10	0.096	0.010	1.174
154	6.89	8.23	66	23.1	2.35	3.14	1.05	0.10	0.251	0.005	1.306
AVE	6.80	8.23	75	25.3	2.51	3.62	1.21	0.07	0.280	0.009	1.447
STD	0.066	0.00	10	10.5	0.35	1.25	0.23	0.02	0.122	0.007	0.196

VS digested (g)	4.54	VS undigested(g)	3.69
Water of Hydrolysis(g)	0.505	**Closure**	**88%**

APPENDIX K

SOLID AND LIQUID TRANSFER DATA FOR COUNTERCURRENT FERMENTATIONS

Table K-1. Liquid volume and solid cake weight in Train A

Fermentation Train A								
	Liquid Volume in Fermentors (mL)				Wet Cake Weight (g)			
Days	F1	F2	F3	F4	F1	F2	F3	F4
96	84	94	96	100	307.8	297.7	295.6	296.5
98	80	96	98	105	311.5	295.7	296.7	292.6
100	84	94	98	98	307.7	299.4	302.3	298.1
102	87	96	101	102	301.9	295.7	293.2	293.9
104	84	99	96	97	306.8	298	299.6	292.7
106	86	95	96	90	307.8	297.4	296.7	290
108	78	90	104	92	310.3	300.2	296.5	300.6
110	82	100	92	101	303.7	298.3	294.1	294.5
112	82	86	94	100	313.3	300.4	302	295.5
114	74	94	98	103	308.3	296.1	297.3	291.2
116	78	92	98	99	310.8	299.4	299	293
118	73	94	98	98	314.5	298.8	294.7	293.7
120	76	96	96	99	312.1	297.2	297.7	296
122	75	90	96	96	316.4	300.6	297.6	295.5
124	74	94	95	98	310.5	297	296.1	296.6
126	84	99	98	102	306.7	290.7	293.2	294.8
128	82	92	103	102	311.7	301.4	293.5	289.2
130	74	98	100	104	313.3	299.9	297.3	292.2
132	77	98	100	92	318.8	299.3	298.6	302.2
134	70	90	86	98	323	302	301.9	295.3
136	76	87	95	108	309.7	292.9	299.1	288.5
138	72	96	104	100	311.8	294.4	300	293.2
140	68	102	98	98	323.4	298.9	297.5	295
142	84	98	94	92	310.3	291.9	298.2	298.8
144	84	94	94	103	311.1	295.2	294.8	291.9
146	86	90	102	92	303.1	294.5	297.3	300.4
148	76	100	90	96	309.1	297.4	296.3	296.8
150	84	88	91	96	309.2	296.5	299.1	297
152	76	88	92	102	309.1	299.2	299.3	291
154	74	90	100	98	308.6	299.5	296.8	296
156	70	93	90	90	314.7	304.2	302	300
					After Subtracting Bottle Weights			
Ave	**79**	**94**	**97**	**98**	**237.0**	**224.2**	**224.7**	**221.5**

Moisture Content (g liquid/g solid cake)		0.59	0.51	0.50	0.48
Volatile Solids (%)		0.21	0.14	0.12	0.12
Holdup (g liquid/g VS cake)	4.07	Total Liquid Weight (g)			841.2
Volatile Solids Concentration (g VS/L)	160	Liquid Residence Time (days)			21

Table K-2. Liquid volume and solid cake weight in Train B

Fermentation Train B								
	Liquid Volume in Fermentors (mL)				Wet Cake Weight (g)			
Days	F1	F2	F3	F4	F1	F2	F3	F4
96	83	104	98	100	318.8	290.8	297	296
98	74	102	93	103	70	110	98	99
100	80	96	98	101	316.2	293.1	299.4	293.6
102	72	102	106	100	320.1	291.4	291.4	296.7
104	81	106	94	100	319.6	293.9	300.3	296
106	84	96	99	97	318.3	292.8	293.2	300.2
108	76	97	100	102	316.5	297	295.2	295.9
110	84	104	96	99	307.5	290.4	299.9	295.4
112	84	98	98	94	314.6	292.9	295.7	300.3
114	76	100	90	98	317.8	293.7	298	297.5
116	84	94	90	96	312.7	290.8	301	299.7
118	76	92	90	96	313.3	293.5	300.4	298.7
120	68	88	96	98	318.9	297.1	295.9	296.7
122	60	96	98	98	323.5	294.2	294	297.8
124	72	96	98	102	318.3	297.6	295.2	294.4
126	70	106	104	101	321	288.2	292.6	294.6
128	78	104	102	96	323	295.5	296	298.4
130	80	106	92	104	320.2	291.7	299.8	293.6
132	68	83	93	94	333.3	303.8	305.4	303
134	58	100	97	100	320.3	288.3	291.9	294.5
136	85	108	100	95	312.9	286.3	295.2	298.5
138	84	104	94	98	318.9	290.9	296.1	296.9
140	78	96	90	95	321.5	293.5	298.4	298.8
142	80	96	90	95	311.3	290.5	299	297.9
144	69	98	96	100	323.1	288.3	293.9	296
146	72	102	100	108	318.8	286.1	292.6	286.6
148	74	102	100	98	300.6	292.6	300.6	291.4
150	80	98	94	88	319	297.1	299.2	304.6
152	70	96	84	92	324.5	291.7	298.9	300.6
154	71	96	90	102	322.4	283.5	299.2	293
156	55	91	90	85	337.4	295.2	306.3	303.2
Ave	**75**	**99**	**95**	**98**	**227.7**	**213.4**	**217.5**	**207.9**

Moisture Content (g liquid/g solid cake)				0.69	0.53	0.50	0.48
Volatile Solids (%)				0.16	0.17	0.12	0.11
Holdup (g liquid/g VS cake)	4.41		Total Liquid Weight (g)				846.1
Volatile Solids Concentration (g VS/L)	145		Liquid Residence Time (days)				23

Table K-3. Liquid volume and solid cake weight in Train C

Fermentation Train C								
	Liquid Volume in Fermentors (mL)				Wet Cake Weight (g)			
Days	F1	F2	F3	F4	F1	F2	F3	F4
96	76	108	100	101	329.5	284.8	296.3	295.6
98	70	110	98	99	335.6	283.9	299.5	295.9
100	84	100	92	92	325.6	293.7	301	301
102	79	99	96	104	316.9	289	294.1	292.6
104	62	97	102	102	332.6	295.6	296.8	293.8
106	86	106	102	102	301.5	292	295.4	292.5
108	100	106	103	94	302.4	293	298.1	295.7
110	96	102	96	106	305.2	296.4	294.6	291.4
112	74	98	105	96	318.9	293.1	296.4	296.8
114	76	100	95	104	319.6	299.4	296.4	291
116	70	90	98	102	326	300.2	300.3	294.2
118	58	96	100	92	328.7	297.3	297.2	300.5
120	61	104	94	104	331.1	291.8	294.1	292.1
122	70	99	99	94	330.1	291.6	298.6	299.7
124	66	104	90	100	328.7	291.4	298.6	296.5
126	86	104	102	106	312.9	281.1	291.7	290.5
128	64	112	104	98	336	286.4	297.8	289.4
130	68	104	92	97	337.4	297.8	301.5	299.1
132	73	98	90	92	327.4	290.5	301.5	303.9
134	78	100	92	100	314.3	286.9	295.4	294.1
136	80	96	100	104	316.4	292	297.1	291.8
138	80	100	104	105	312.4	295.7	296.4	291.2
140	76	100	101	98	320.3	298.6	300.3	294.2
142	73	102	96	90	322.9	294.1	300.7	300.9
144	73	104	88	96	324.4	287.3	297.1	298.7
146	66	100	96	98	332.4	284.1	292.7	294.6
148	64	104	94	93	327.9	287.9	299.4	300.7
150	64	96	84	96	336.5	292.1	304.4	298.9
152	68	96	92	100	327	284.8	298.3	296.2
154	66	106	100	98	331.6	284	297	296.5
156	60	102	90	94	342.1	292.5	306.2	302.2
Ave	**73**	**101**	**97**	**99**	**250.1**	**217.9**	**224.2**	**222.5**

Moisture Content (g liquid/g solid cake)			0.69	0.57	0.53	0.52
Volatile Solids (%)			0.17	0.17	0.14	0.12
Holdup (g liquid/g VS cake)	4.31	Total Liquid Weight (g)				900
Volatile Solids Concentration (g VS/L)	150	Liquid Residence Time (days)				25

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