CONVERSION OF SUGARCANE BAGASSE TO CARBOXYLIC ACIDS UNDER THERMOPHILIC CONDITIONS

A Dissertation

by

ZHIHONG FU

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

DOCTOR OF PHILOSOPHY

May 2007

Major Subject: Chemical Engineering

CONVERSION OF SUGARCANE BAGASSE TO CARBOXYLIC ACIDS UNDER THERMOPHILIC CONDITIONS

A Dissertation

by

ZHIHONG FU

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

DOCTOR OF PHILOSOPHY

Approved by:

Chair of Committee,	Mark T. Holtzapple
Committee Members,	Richard R. Davison
	Charles J. Glover
	Cady R. Engler
Head of Department,	N.K. Anand

May 2007

Major Subject: Chemical Engineering

ABSTRACT

Conversion of Sugarcane Bagasse to Carboxylic Acids under Thermophilic Conditions. (May 2007)

Zhihong Fu, B.S.; M.S., Xiamen University, PR China

Chair of Advisory Committee: Dr. Mark T. Holtzapple

With the inevitable depletion of the petroleum supply and increasing energy demands in the world, interest has been growing in bioconversion of lignocellulosic biomass (e.g., sugarcane bagasse). Lignocellulosic biomass is an abundant, inexpensive, and renewable resource. Most of current conversion technologies require expensive enzymes and sterility. In contrast, the patented MixAlco process requires no enzymes or sterility, making it attractive to convert lignocellulosic biomass to transportation fuels and valuable chemicals. This study focuses on pretreatment and thermophilic fermentation in the MixAlco process.

Ammonium bicarbonate (NH_4HCO_3) was discovered to be a better pH buffer than previously widely used calcium carbonate ($CaCO_3$) in anaerobic fermentations under thermophilic conditions (55°C). The desired pH should be controlled within 6.5 to 7.5. Over 85% acetate content in the product was found in paper fermentations and bagasse fermentations. Hot-lime-water-treated bagasse countercurrent fermentations buffered by ammonium bicarbonate achieved 50–60% higher total product concentrations than those using calcium carbonate. It was nearly double in paper batch fermentations if the pH was controlled around 7.0.

Ammonium bicarbonate is a “weak” methane inhibitor, so a strong methane inhibitor (e.g., iodoform) is still required in ammonium bicarbonate buffered fermentations. Residual calcium salts did not show significant effects on ammonium bicarbonate buffered fermentations.

Lake inocula from the Great Salt Lake, Utah, proved to be feasible in ammonium bicarbonate buffered fermentations. Under mesophilic conditions (40°C), the inoculum from the Great Salt Lake increased the total product concentration about 30%, compared to the marine inoculum. No significant fermentation performance difference, however, was found under thermophilic conditions.

The Continuum Particle Distribution Model (CPDM) is a powerful tool to predict product concentrations and conversions for long-term countercurrent fermentations, based on batch fermentation data. The experimental acid concentrations and conversions agree well with the CPDM predictions (average absolute error < 15%).

Aqueous ammonia treatment proved feasible for bagasse. Air-lime-treated bagasse had the highest acid concentration among the three treated bagasse. Air-lime treatment coupled with ammonium bicarbonate buffered fermentations is preferred for a "crop-to-fuel" process. Aqueous ammonia treatment combined with ammonium bicarbonate buffered fermentations is a viable modification of the MixAlco process, if "ammonia recycle" is deployed.

DEDICATION

I dedicate this dissertation to my wonderful wife, Jing Chen. This work would not have been possible without her continuous love and support.

ACKNOWLEDGEMENTS

My gratitude goes to my academic advisor, Dr. Mark T. Holtzapple, for his guidance and generous financial support. It is impossible to complete this work without his continuous inspiration, encouragement, and support. Working with him is not only an honor, but also a wonderful experience of a lifetime that I will cherish forever. His dedication to teaching, research, and engineering has set the standard I will look up to in my whole life. I will never forget his dreams: "Imagine climbing into your car in California and driving to New York — without stopping once to fill the fuel tank." His concepts of "90-miles-per-gallon car" and "Crop-to-Wheel" will always drive me in my future career.

I express my appreciation to the members of my committee, Dr. Richard Davison, Dr. Charles J. Glover, and Dr. Cady Engler, for their time reading this dissertation and for their valuable comments. I thank my group members, Cesar Granda, Frank Agbogbo, Li Zhu (Julie), Jonathan O'Dwyer, Sehoon Kim, Cateryna Aiello-Mazzarri, Guillermo Coward-Kelly, Wenning Chan, Piyarat Thanakoses, Xu Li, Maxine Jones, Stanley Coleman, Rocio Sierra, Andrea Forrest, Aaron Smith, Somsak Watanawanavet, Andrew Moody, Nicolas Rouckout, and Randy Miles, for all their support and encouragement. I would like to specifically thank Frank Agbogbo for continuous help and encouragement when overcoming "fermentation" puzzles. My appreciation also goes to all student workers who worked in our laboratory for the past several years. The experimental work in this dissertation was difficult, challenging, and time-consuming. Without the student workers' help, the over 4,500 experimental points in this dissertation would have been an impossible mission.

I would like to express my special appreciation to Dr. Rayford Anthony for his support and substitution for Dr. Glover when Dr. Glover was not available for my preliminary exam. Also, appreciation is extended to Towanna Mann, Ninnete Portales,

Missy Newton, and Randy Marek, staff members in the Artie McFerrin Department of Chemical Engineering. They have provided all kinds of help during my study in Texas A&M University. I am also thankful to the friendship developed with many of other faculty and staff members. Their support and encouragement will always be in my heart.

TABLE OF CONTENTS

CHAPTER Page

Page

LIST OF FIGURES

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

FIGURE Page

LIST OF TABLES

TABLE Page

TABLE Page

TABLE Page

CHAPTER I
INTRODUCTION

Biomass is a sustainable, renewable, but underdeveloped resource. Biomass conversion not only provides heat, electricity, and biofuels, but also reduces carbon dioxide emissions and therefore prevents global warming. In this chapter, the current status of biomass conversion technologies is reviewed. This is followed by introducing promising lignocellulosic biomass feedstocks and challenges in lignocellulosic biomass conversion. Subsequently, it presents the process description and recent advances of the MixAlco process, a novel and promising biomass conversion technology to convert biomass into chemicals and fuels. The last part summarizes the objectives and rationale of this dissertation.

1.1 Biomass conversion technology

Biomass is a term describing organic material from plants. Biomass sources are diverse and include agricultural wastes (e.g., corn stover and sugarcane bagasse), forest residues, industrial wastes (e.g., sawdust and paper pulp), as well as energy crops (e.g., sorghum and energy cane). As illustrated in Figure 1-1, plant materials use solar energy to convert atmospheric carbon dioxide to sugars during photosynthesis. Once biomass is combusted, energy is released as the sugars are converted back to carbon dioxide. Therefore, biomass energy is close to "carbon neutral," that is, it produces energy by releasing carbon to the atmosphere that was captured during plant growth.

This dissertation follows the style of Biotechnology and Bioengineering.

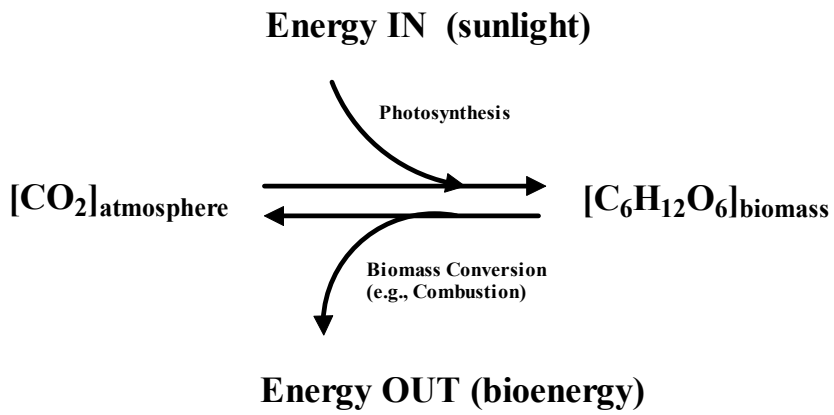


Figure 1-1. Conceptual flowchart of biomass conversion.

Biomass has always been a major source of energy for mankind. For centuries, biomass was combusted for heating and cooking. Even today, biomass contributes significantly to the world's energy supply. In the future, its use is expected to grow due to the inevitable depletion of the world's petroleum supply and increasing energy demands. Bioenergy is one of the key options to mitigate greenhouse gas emissions and to substitute for fossil fuels (Goldemberg 2000). Biomass also has great potential to provide heat and power to industry and to provide feedstocks to make a wide range of chemicals and materials (bioproducts). In the 21st century, biomass is expected to contribute 200–300 EJ energy annually, which makes biomass an important and promising energy supply option in the future (Faaij 1999).

Figure 1-2 shows the main biomass conversion technologies that are used or under development for producing heat, electricity, and transportation fuels. In Section 1.1.1, conversion technologies for producing power and heat will be summarized (combustion, gasification, pyrolysis, and digestion). Section 1.1.2 describes the technologies for producing transportation fuels (fermentation, gasification, and extraction).

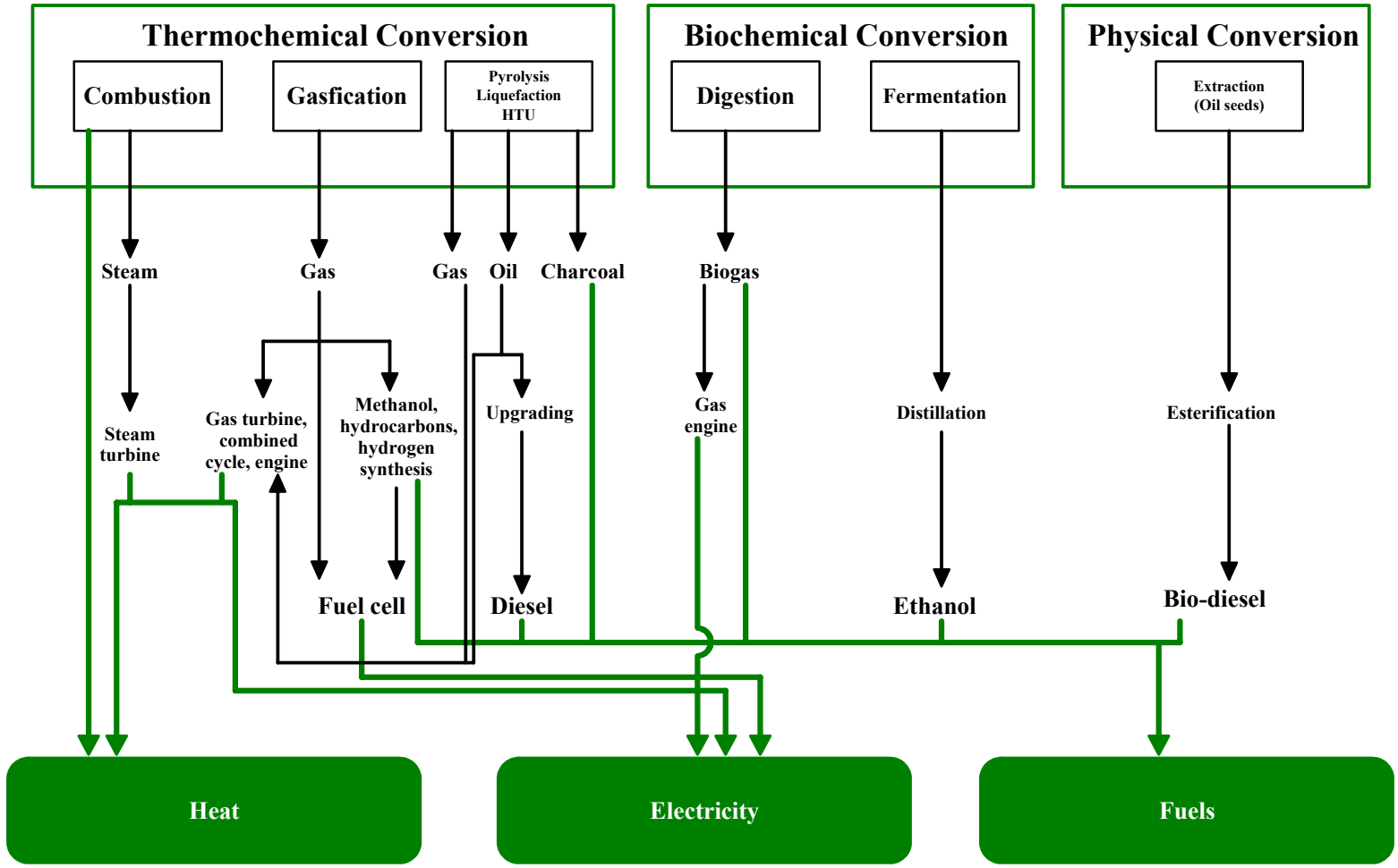


Figure 1-2. Main conversion technologies for biomass to energy (Turkenburg 2002).

1.1.1 Combustion, gasification, pyrolysis, and digestion for power and heat

Combustion

Combustion is the dominant biomass conversion technology. Production of heat (domestic and industrial) and electricity (i.e., combined heat and power) is the main route (Figure 1-2). A classic application of biomass combustion is heat production for domestic applications. Also, combustion of biomass for electricity production (plus heat and process steam) is applied commercially word wide. Co-firing of coal and biomass effectively controls NO_x emission from coal combustion (Backreedy et al. 2005; Demirbas 2003; Demirbas 2005; Lee et al. 2003).

Gasification

Gasification is another method to convert diverse solid fuels to combustible gas or syngas (i.e., CO and H_2). Gasification converts biomass into fuel gas, which can be further converted or cleaned prior to combustion (e.g., in a gas turbine). When integrated with a combined cycle, this leads to a BIG/CC (Biomass Integrated Gasification/Combined Cycle plant). Gasification of dry biomass has a higher conversion efficiency (40–50%) than combustion, and generates electricity through a gas turbine. Development of efficient BIG/CC systems with 5–20 MWe capacity are nearing commercial realization, but the challenges of gas clean-up remain (Dowaki et al. 2005; Kumar et al. 2003; Turn 1999).

Production of bio-oils: Pyrolysis and liquefaction

Pyrolysis is an important thermal conversion process for biomass. Up to now, pyrolysis is less developed than gasification. Major attention was especially caused by the potential deployment of this technology on small scale in rural areas and as feedstock for the chemical industry. Pyrolysis converts biomass at temperatures around 500°C in the absence of oxygen to liquid (bio-oil), gaseous, and solid (char) fractions (Adjaye et al. 1992; Demirbas and Balat 2006; Miao and Wu 2004; Zhang et al. 2007). With flash

pyrolysis techniques (fast pyrolysis), the liquid fraction (bio-oil) can be maximized up to 70 wt% of the biomass input. Crude bio-oil can be used for firing engines and turbines. The bio-oil may also be upgraded (e.g., via hydrogenation) to reduce the oxygen content. Liquefaction (conversion under high pressure) and HTU (i.e., Hydro Thermal Upgrading) are other ways of producing 'raw intermediate' liquids from biomass. HTU is a promising process originally developed by Shell and is in the pre-pilot phase. It converts biomass to bio-crude at a high pressure in water and moderate temperatures (Naber 1997).

Digestion

Anaerobic digestion of biomass to produce biogas is another route to fuels. Anaerobic digestion is particularly suitable for wet biomass materials. This has been demonstrated and applied commercially with success for various feedstocks, including organic domestic waste, organic industrial wastes, and manure (Hansen et al. 2006; Mao and Show 2006; Murphy and Power 2006; Nguyen et al. 2007). Digestion has been deployed for a long time in the food and beverage industry to process waste water with high organic loading (Moletta 2005; Stabnikova et al. 2005). Conversion of biomass to gas can reach about 35%, but strongly depends on the feedstock. It has a low overall electrical efficiency when the gas is used in engine-driven generators (typically 10–15%).

Landfill gas utilization (DeJager and Blok 1996; Gardner et al. 1993; Lagerkvist 1995; Murphy et al. 2004) is another specific source for biogas. The production of methane-rich landfill gas from landfill sites makes a significant contribution to atmospheric methane emissions. In many situations, the collection of landfill gas and production of electricity by converting this gas in gas engines is profitable and feasible. Landfill gas utilization is attractive, because it prevents the build-up of methane in the atmosphere, which has a stronger "greenhouse" impact than CO_2.

1.1.2 Gasification, extraction, and fermentation for transportation fuel production

As illustrated in Figure 1-1, three major routes can be deployed to produce transportation fuels from biomass. Gasification can be used to produce syngas, which can be further converted to methanol, Fischer-Tropsch liquids, dimethylether (DME), and hydrogen. Biofuels can be produced via extraction from oil seeds (e.g., rapeseed), which can be esterified to produce biodiesel. Finally, ethanol production can occur via direct fermentation of sugar- and starch-rich biomass, the most utilized route for production of biofuels to date. Table 1-1 compares some major properties of the traditional transportation fuel and novel biofuels.

Table 1-1. Some major properties of traditional fuels and biofuels (Castro et al. 2003; Gordon and Austin 1992; Maclean 2004; Steinberg 1999).

Fuel	Density (kg/L, at 15°C)	Energy density (MJ/kg)	Other aspects
Hydrogen	0.07	142	Lighter than air; explosion limits 4.00–74.20%
Methanol	0.8	23	Toxic in direct contact; octane number 88.6 (gasoline 85)
DME	0.66	28.2	Vapor pressure 5.1 bar at 20°C.
Fischer-Tropsch gasoline	0.75	46–48	Very comparable to diesel and gasoline; zero sulfur; no aromatics
Ethanol	0.79	30	Nontoxic, biodegradable; octane number 89.7 (gasoline 85)
Diesel from bio-oil/bio-crude	0.85	47	Fully deoxygenated
Bio-diesel	0.88	42	
Gasoline	0.75	46	Depending on refining process, contains sulfur and aromatics
Diesel	0.85	46	Depending on refining process, contains sulfur and aromatics

Methanol, hydrogen and hydrocarbons via gasification

Figure 1-3 shows biomass can be converted into methanol, hydrogen, and Fischer-Tropsch diesel via gasification. All routes need very clean syngas before the secondary energy carrier is produced via relatively conventional gas processing methods. Besides Methanol, hydrogen and FT-liquids, DME (dimethylether) and SNG (Synthetic Natural Gas) can also be produced from syngas.

Extraction and production of esters from oilseeds

Extraction is a mechanical conversion process, which can be used to obtain oil from oilseed. Vegetable oils used as an alternative fuel for Diesel engines are gaining an increasing interest in agriculture, electricity generation, and transportation. Oilseeds (e.g., rapeseed) can be extracted and converted to esters, which are suitable to replace diesel (Karaosmanoglu 2000; Ozcimen and Karaosmanoglu 2004). This process is used commercially on a substantial scale, especially in Europe. Cotton oil (Vaitilingom 2006), camelina oil (Bernardo et al. 2003), and rapeseed oil (Culcuoglu et al. 2002) have been studied. For a typical rapeseed extraction, the process produces not only oil but also rapeseed cake, which is suitable for fodder. Rapeseed oil can then be esterified to obtain rapeseed methyl ester (RME) or bio-diesel.

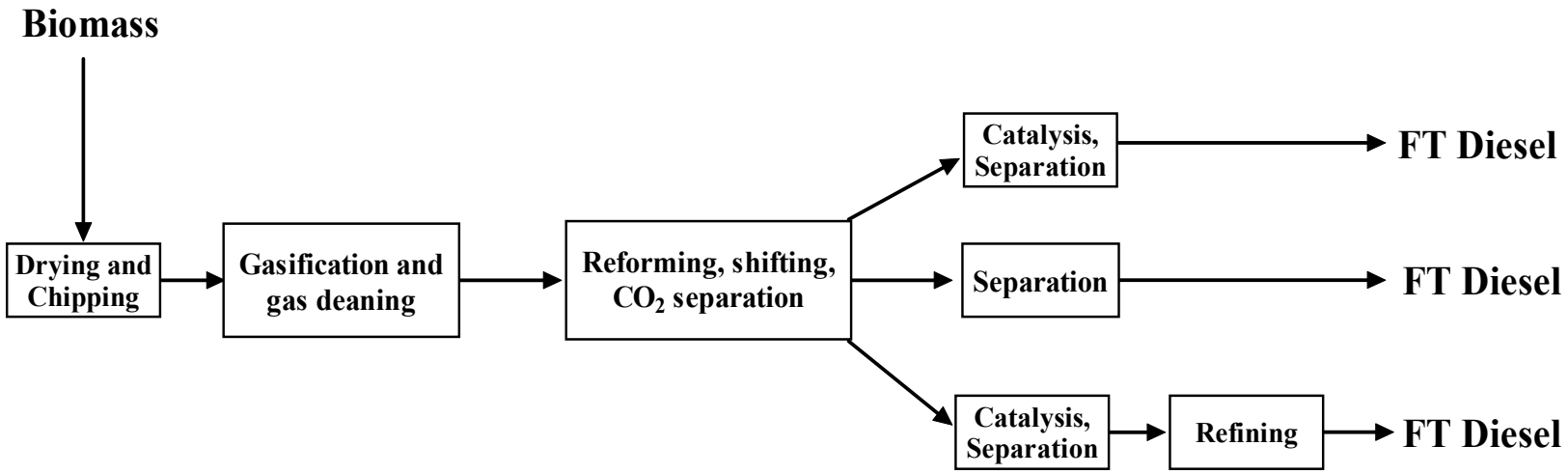


Figure 1-3. General flowchart for biomass gasification to produce methanol, hydrogen, and FT diesel.

Ethanol via fermentation

By far, ethanol is the most wildly used biofuel. Ethanol can serve as standalone fuel or blended with gasoline. There are 111 ethanol refineries nationwide, with the capacity to produce more than 5.4 billion gallons annually (Mufson 2007). In 2007, there are 78 ethanol refineries and eight expansions under construction with a combined annual capacity of more than 6 billion gallons.

Ethanol fermentation is a mature commercial technology. Large-scale application of modern fermentation involves conversion of sugar and starch utilization (Lin and Tanaka 2006). Sugars (from sugarcane, sugar beets, molasses, and fruits) can be converted into ethanol directly. Starches (from corn, cassava, potatoes, and root crops) must first be hydrolyzed to fermentable sugars by the action of enzymes from malt or molds. The conversion of starch to ethanol includes a liquefaction step (to make starch soluble) and a hydrolysis step (to produce glucose). Once simple sugars are formed, enzymes from microorganisms can readily ferment them to ethanol. Future fermentation processes (Figure 1-4) are proposed to convert lignocellulosic biomass to ethanol.

Current fermentation technology is subject to the high costs associated with grain feedstock (e.g., corn), year-to-year volatility of the grain market, and expensive enzymes. Also, current available microorganisms cannot efficiently ferment five-carbon (pentoses) sugars.

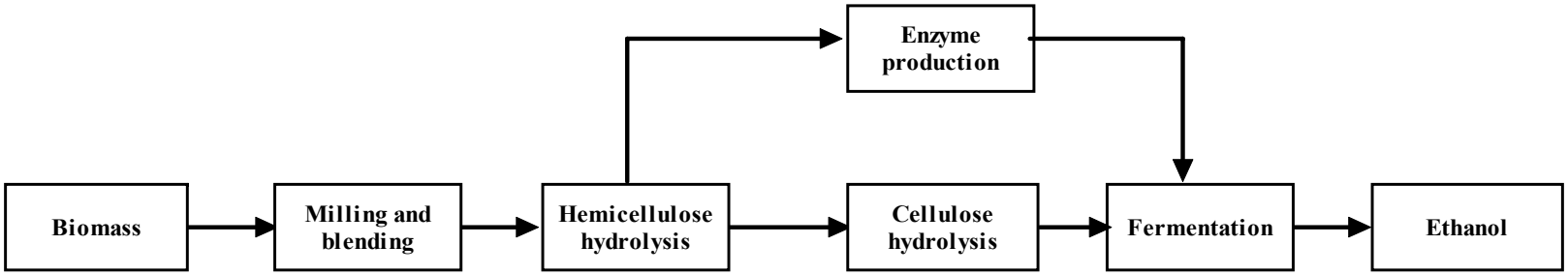


Figure 1-4. Overview of ethanol production by fermentation technology.

1.2 Utilization of lignocellulosic biomass

With oil prices soaring, growing security risks of petroleum dependence, and the environmental costs of fossil fuels, biomass is an attractive alternative because it is the only current renewable source of liquid transportation fuel. As mentioned in Section 1.1.2, commercial transportation biofuel from biomass is ethanol derived from corn grain (starch) and sugarcane (sucrose). However, both biomass feedstocks are expensive, compete with food, and are expected to be limited in supply in the near future. In summary, biomass availability, biomass feedstock cost, and biomass conversion technology are major bottlenecks for biofuels to be cost-competitive with fossil fuels.

Lignocellulosic biomass is regarded as the most attractive, promising, and substantial feedstock for transportation fuel (i.e., lignocellulosic ethanol). Compared with corn and cane, lignocellulosic biomass is an abundant and inexpensive resource that accounts for approximately 50% of the biomass in the world, but still is not commercially developed. Annual lignocellulosic biomass production is estimated to be 10–50 billion t (Claassen et al. 1999); therefore, utilization of lignocellulosic biomass can open a new window towards low-cost and efficient production of transportation fuels.

1.2.1 Chemical structure of lignocellulosic biomass

Unlike starch, which contains homogeneous and easily hydrolyzed polymers, lignocellulose biomass contains cellulose (23–53%), hemicellulose (20–35%), lignin (10–25%), and other possible extractable components (Himmel et al. 1997; Knauf and Moniruzzaman 2004). The first three components contribute most of the total mass and are the major problem for biomass conversion. The chemical properties of cellulose, hemicellulose, and lignin are therefore detailed in the following section:

Cellulose

Cellulose is a major component of primary and secondary layers of plant cell walls. It is found as microfibrils (2–20 nm diameter and 100–40,000 nm long), which form the structurally strong framework in the cell walls. Cellulose is a linear polymer of 1,000 to 10,000 β-(1→4)-D-glucopyranose units (Figure 1-5). The fully equatorial conformation of β-linked glucopyranose residues stabilizes the chair structure, minimizing its flexibility. By forming intramolecular and intermolecular hydrogen bonds between OH groups within the same cellulose chain and the surrounding cellulose chains, the chains tend to arrange in parallel and form a crystalline supermolecular structure. Then, bundles of linear cellulose chains (in the longitudinal direction) form a microfibril that is a component of the cell wall structure.

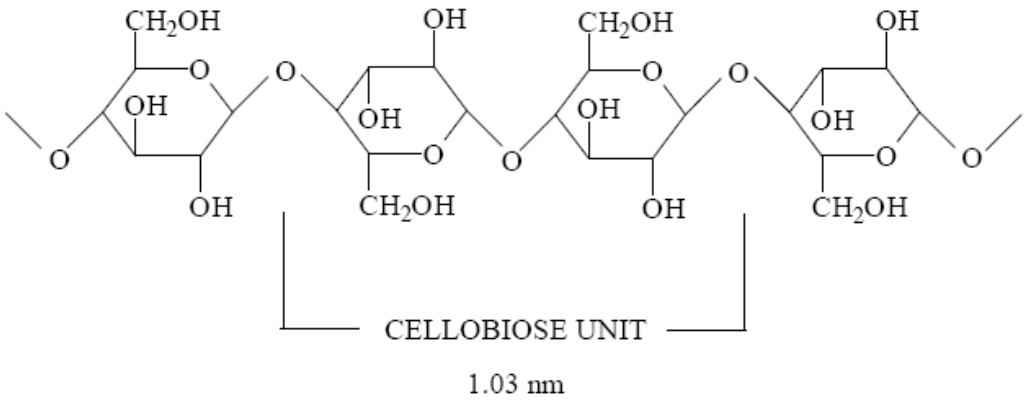


Figure 1-5. Schematic illustration of the cellulose chain.

Hemicellulose

Hemicellulose is abundant in primary plant cell walls, but is also found in secondary walls. Hemicellulose is a polysaccharide composed of various sugars including xylose, arabinose, and mannose. Unlike cellulose, hemicelluloses consist of

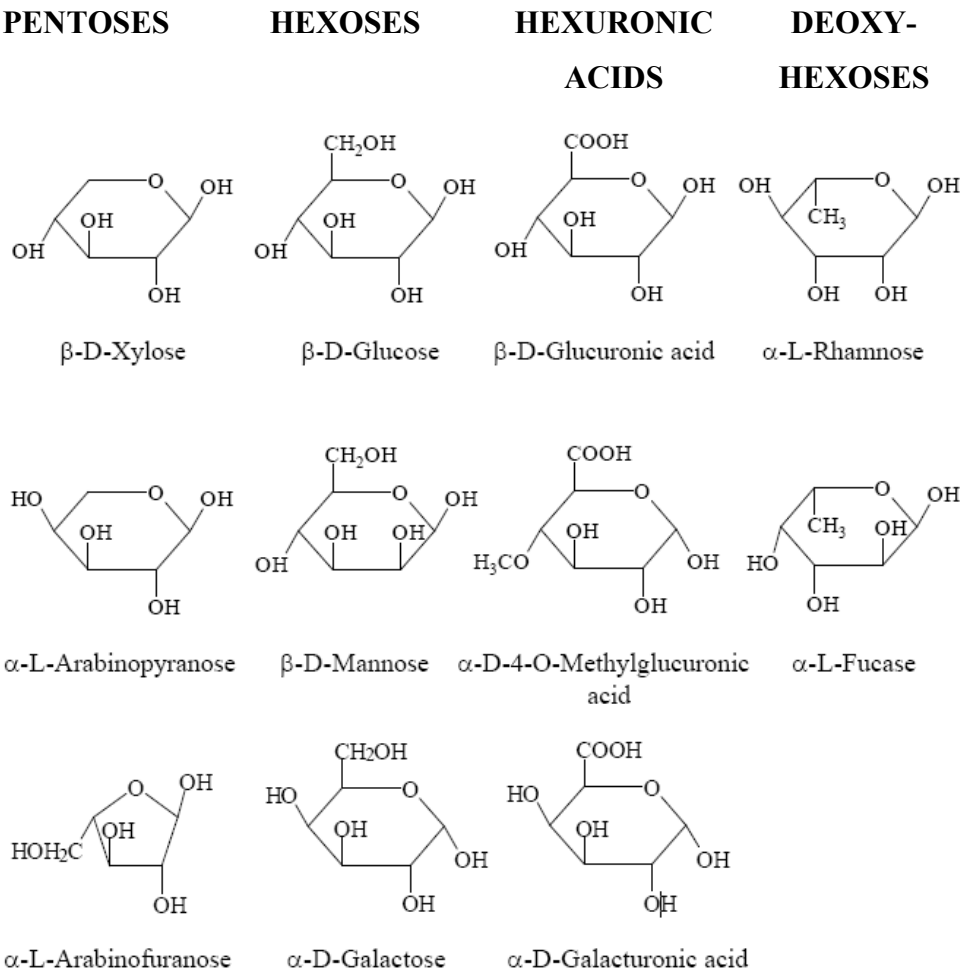


Figure 1-6. Schematic illustration of sugar units of hemicelluloses.

different monosacharide units. In addition, the polymer chains of hemicelluloses have short branches and are amorphous. Because of their amorphous morphology, hemicelluloses are partially soluble or swellable in water. The backbone of a hemicellulose chain can be a homopolymer (generally consisting of single sugar repeat unit) or a heteropolymer (mixture of different sugars). Formulas of the sugar components of hemicelluloses are listed in Figure 1-6.

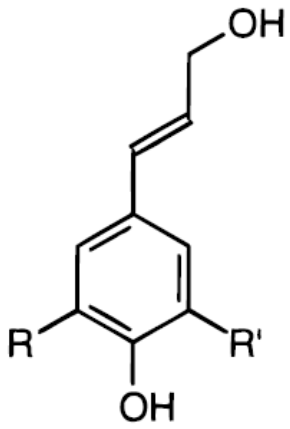


Figure 1-7. Schematic illustration of building units of lignin: p-hydroxyphenyl unit (R = R' = H, guaiacyl unit (R = H, R' = OCH_3), and syringyl units (R = R' = OCH_3).

Hemicellulose that is primarily xylose or arabinose is referred to as xyloglucans or arabinoglucans, respectively. Hemicellulose molecules are often branched. Like the pectic compounds, hemicellulose molecules are very hydrophilic.

Lignin

Lignin is a complex, crosslinked polymer that reinforces the walls of certain cells in higher plants. Lignin gives mechanical strength to plant by gluing the fibers together (reinforcing agent) between the cell walls. It is mainly found in the vascular tissues, where its hydrophobicity waterproofs the conducting cells of the xylem and its rigidity strengthens the supporting fiber cells of both the xylem and phloem. It may also play an important role in defense against pathogen attack (Hawkins et al. 1997). The monomeric building units of lignin are p-hydroxyphenyl, guaiacyl, and syringyl units (Figure 1-7).

1.2.2 Challenges of lignocellulosic biomass

Although lignocellulosic feedstock is available in large quantities, the main challenge for commercialization is to reduce the operating costs of biomass conversion processes, primarily pretreatment and enzymes (Gnansounou and Dauriat 2005; Kamm and Kamm 2004; Tengerdy and Szakacs 2003; Van Groenestijn et al. 2006; Zaldivar et al. 2005).

Efficient and cost-effective pretreatment technology

Most biomass pretreatment methods do not hydrolyze significant amounts of the cellulose fraction of biomass. Pretreatment enables more efficient enzymatic hydrolysis of the cellulose by removing the surrounding hemicellulose and/or lignin along with modifying the cellulose microfiber structure. Although the resulting composition of the treated biomass depends on the biomass feedstock and pretreatment methods, it is generally much more amenable to enzymatic digestion than the original biomass. A universal pretreatment process is difficult to develop due to the diverse nature of biomass. The general criteria for a successful biomass pretreatment can be narrowed to high cellulose digestibility, high hemicellulose sugar recovery, low capital and energy cost, low lignin degradation, and recoverable process chemicals.

Advanced enzymes for efficient biomass hydrolysis

The major bottleneck for ethanol production from lignocellulosic biomass lies in enzymatic hydrolysis of cellulose using cellulase enzymes. Cellulases are slow enzymes primarily because of the complex, insoluble, and semicrystalline nature of their substrate. In addition, maximal cellulase activity requires multiple, related enzymes such as endogluconases, exogluconases, and beta-glucosidases to act synergistically for complete conversion of cellulose into glucose. Currently, the expense of cellulase and related enzymes make lignocellulosic biomass processing uncompetitive with corn or sugarcane, even after decades of research in improving cellulase enzymes. The engineering of cellulase enzymes for lignocellulosic biomass processing therefore faces

various challenges. Advances are needed in stability, yield, and specific activity. They also need to be effective in harsh environments generated by biomass pretreatment processes.

Efficient fermentation of pentose sugars

The glucose produced from cellulose hydrolysis can be easily fermented with existing microorganisms. However, hydrolysis of hemicellulose from biomass produces both hexose (C6) and pentose (C5) sugars (i.e., mannose, galactose, xylose, and arabinose), which cannot be efficiently handled by existing microorganisms. Optimized microorganisms and processes are necessary to ferment these "unusual" sugars, especially pentoses. Genetically modified fermentation microorganisms such as *Saccharomyces, E. coli*, and *Zymomonas* that can utilize C5 sugars have been developed. Researchers have also tried to develop microbial process that can simultaneously hydrolyze and ferment amorphous cellulose. Such advanced ethanol-producing microorganisms can secret endoglucanases along with utilizing dimers and trimers of glucose and xylose, and metabolize C5 sugars. But, ethanol yields from either genetically modified microorganisms or microbial processes are still not sufficient to make pentose sugar fermentation economically attractive.

In conclusion, current commercial biomass-to-fuel conversion technology is enzyme-based. For example, SSF process (simultaneous saccharification and fermentation) gives high reported ethanol yields but requires expensive enzyme and strict fermentation conditions, including sterility (Dien et al. 2003). The other challenge for current enzymes is to efficiently handle pentose sugars (C5). In contrast, the MixAlco process (Section 1.3) requires no enzymes or sterility, making it an attractive alternative to convert lignocellulosic biomass into transportation fuels and valuable chemicals. Furthermore, the MixAlco process can use all biodegradable components in biomass.

1.3 The MixAlco process

The MixAlco process (Domke et al. 2004; Holtzapple et al. 1999; Holtzapple et al. 1997; Thanakoses et al. 2003) is well-developed, has received over 10 U.S. patents (Table 1-2) and numerous pending patents, and is ready for commercialization. A pilot plant with capacity of 100 lb/day is operating in College Station, TX (Figure 1-8). This process utilizes biological/chemical methods to convert any biodegradable material (e.g., municipal solid waste, biodegradable waste, and agricultural residues such as sugarcane bagasse) into valuable chemicals (e.g., carboxylic acids and ketones) and fuels, such as a mixture of primary alcohols (e.g., ethanol, propanol, and butanol) and a mixture of secondary alcohols (e.g., isopropanol, 2-butanol, and 3-pentanol).

Table 1-2. Awarded patents to the MixAlco process.

U.S. Patent number	Patent title	Patent awarded date
5,693,296	Calcium hydroxide pretreatment of biomass	December 2, 1997
5,865,898	Methods of biomass pretreatment	February 2, 1999
5,874,263	Method and apparatus for producing organic acids	February 23, 1999
5,962,307	Apparatus for producing organic acids	October 5, 1999
5,969,189	Thermal conversion of volatile fatty acid salts to ketones	October 19, 1999
5,986,133	Recovery of fermentation salts from dilute aqueous solutions	November 16, 1999
6,043,392	Method for conversion of biomass to chemicals and fuels	March 28, 2000
6,262,313	Thermal conversion of fatty acid salts to ketones	July 17, 2001
6,395,926	Process for recovering low boiling acids	May 28, 2002
6,478,965	Recovery of fermentation salts from dilute aqueous solutions	November 12, 2002

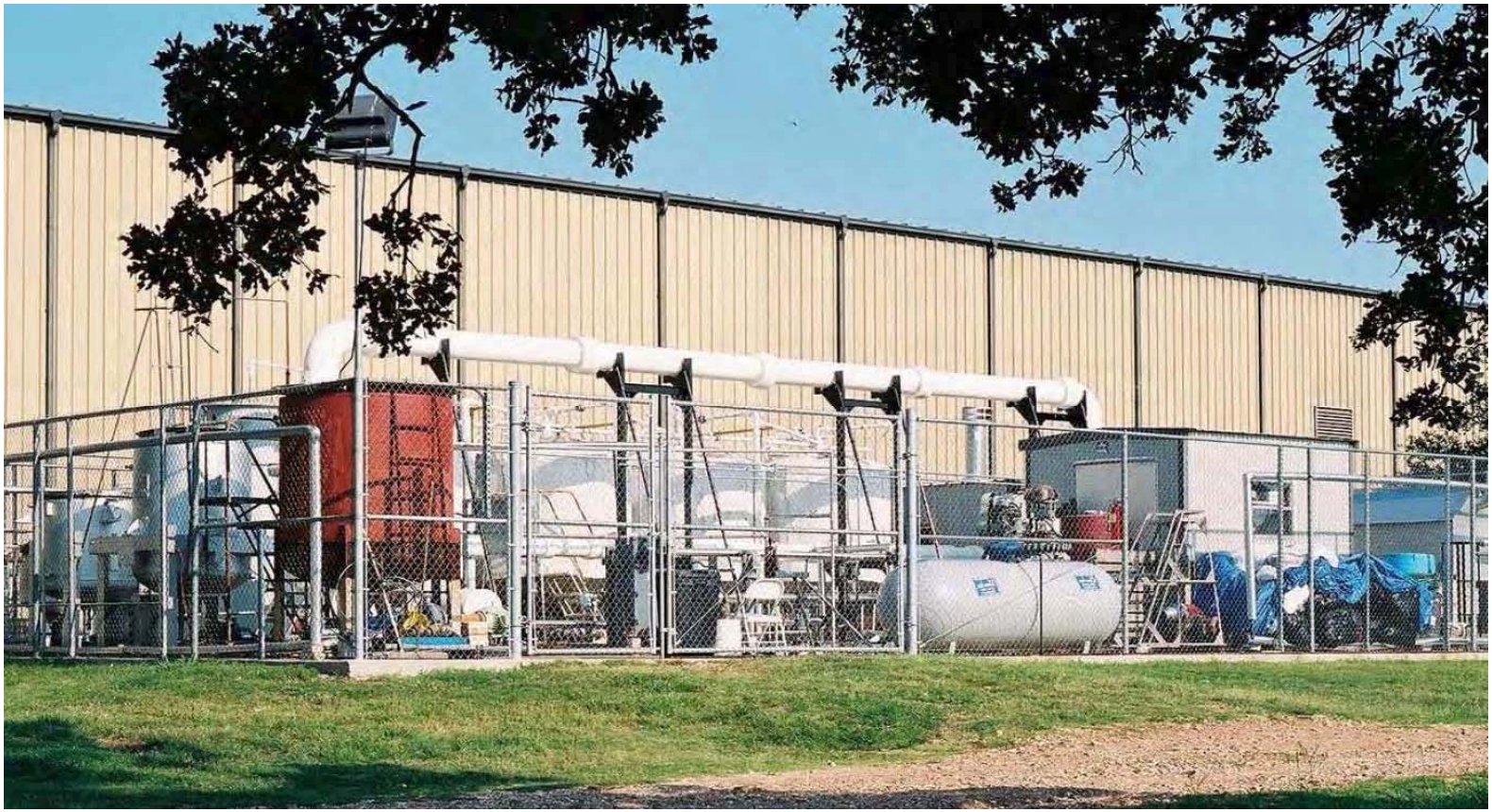


Figure 1-8. Photograph of the MixAlco process pilot plant in College Station, TX.

1.3.1 Description of the MixAlco process

Figure 1-9 summarizes the MixAlco process (Holtzapple et al. 1999; Holtzapple et al. 1997) for converting biomass into chemicals and fuels. Biomass is pretreated with lime to enhance digestibility, and then is fermented anaerobically using a mixed culture of carboxylic acid-forming microorganisms. A buffer is added to neutralize the produced acids and maintains a desired pH range in the fermentation broth. The resulting carboxylate salt solution is concentrated. The concentrated carboxylate salts can be converted to carboxylic acids by acid springing. The acids can be catalytically converted to ketones, which are further converted into mixed secondary alcohols (e.g., isopropanol) by hydrogenation. Alternatively, the concentrated acids can be esterified and then hydrogenated to mixed primary alcohols (e.g., ethanol). Both carboxylic acids and ketones, intermediate product in the MixAlco process, are valuable chemicals and could be sold as desired products.

Pretreatment

Because lime ($Ca(OH)_2$) is inexpensive and easy to handle, lime treatment is the first choice in the MixAlco process. Lime treatment has been used to pretreat various biodegradable materials including switchgrass (Chang et al. 1997), corn stover (Kim and Holtzapple 2005; Kim and Holtzapple 2006a; Kim and Holtzapple 2006b), poplar wood (Chang et al. 2001), and sugarcane bagasse (Chang et al. 1998; Gandi et al. 1997). In the case of herbaceous materials, effective lime treatment conditions are 100°C for 1–2 h with a lime loading of 0.1 g $Ca(OH)_2$/g biomass. The pretreatment is not affected by water loading; 5–15 g H_2O/g biomass is effective provided mixing is adequate. In the case of high-lignin biomass, combination lime treatment with pressurized oxygen (1.5 MPa) is effective (Chang et al. 2001), although pretreatment costs increase due to the required pressure vessel for high-pressure oxygen.

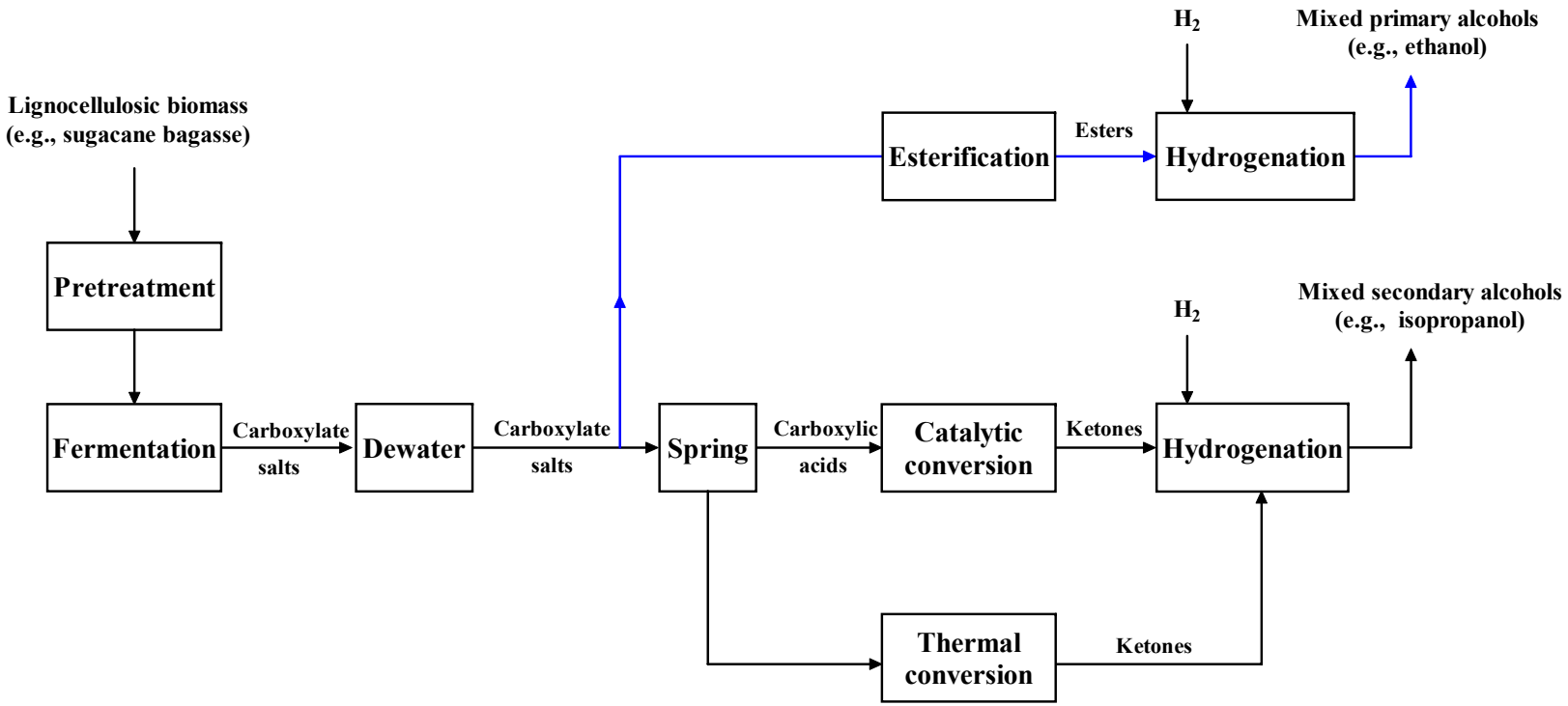


Figure 1-9. Overview of the MixAlco process.

Anaerobic fermentation

Anaerobic fermentations use a mixed culture of natural microorganisms found in habitats such as the rumen of cattle, termite guts, and terrestrial swamps to anaerobically digest biomass into a mixture of carboxylic acids. No sterility is required. The operating temperature can be 40°C (mesophilic condition) or 55°C (thermophilic condition) (Agbogbo 2005; Aiello Mazzarri 2002; Thanakoses 2002). The preferred feedstock is 80 wt% carbon source (e.g., sugarcane bagasse) and 20 wt% nutrient source (e.g., chicken manure). As the microorganisms anaerobically digest the biomass and convert it into a mixture of carboxylic acids, the pH must be controlled. This is done by adding a buffering agent (e.g., calcium carbonate), thus yielding a mixture of carboxylate salts.

Dewatering

The acid concentration in the fermentation broth typically is 30–50 g/L; therefore, dewatering of this dilute solution is necessary. Amine dewatering technology was previously used to dewater the fermentation broth. Currently, a vapor-compression evaporator is used to remove most of the water (over 90%). Vapor-compression evaporators utilize mechanical power to pressurize the evaporated steam. Then, this pressurized steam is sent to a heat exchanger where it provides the latent heat of vaporization for more water to be evaporated. The efficiency of this vapor compression evaporator is equivalent to 40–80 effects of a multi-effect evaporator (Granda and Holtzapple 2006).

Acid spring

The carboxylic acids can be recovered using an "acid springing" process. The concentrated salts are contacted with a high-molecular-weight (HMW) tertiary amine (e.g., trioctylamine). The resulting amine carboxylate is heated to "spring" or release the acids in a reactive distillation column. The carboxylic acids are harvested at the top, whereas the HMW tertiary amine is recovered at the bottom and recycled back to react

with the fresh concentrated salts from the dewatering process. In theory, no HMW tertiary amine is consumed in this process.

Esterification and hydrogenation

The ester-alcohol path is applied if the desired product is primary alcohols (e.g., ethanol). The concentrated salt solution is contacted with a high-molecular-weight alcohol (e.g., heptanol) in the presence of acid catalyst (e.g., zeolites) to yield esters (e.g., heptyl acetate). The resulting esters are hydrogenated in the presence of a catalyst (e.g., Raney nickel) and then sent to a distillation column to separate the products. Hydrogen can be obtained from many sources, such as gasification of the undigested residue from the fermentation. The ester hydrogenation follows:

$$RCOOR' + 2\ H_2 \rightarrow R\text{-}CH_2OH + R'OH$$

Ketone production and hydrogenation

The ketone-alcohol path is used to produce secondary alcohols (e.g., isopropanol). When calcium carboxylate salts are preheated to around 430°C, the salts will decompose to ketones with a reported yield as high as 99.5%. At 430°C, the half-life of the reaction is less than 1 min; therefore the reaction is very rapid. The reaction temperature has no effect on ketone quality in range of 430–508°C. Alternatively, ketones can be produced by passing carboxylic acids over a catalyst (e.g., zirconium oxide) using gas-phase catalytic conversion. The resulting ketones are heated and introduced to a hydrogenation reactor. The ketones are hydrogenated in the presence of a catalyst (e.g., platinum). Hydrogen can be obtained from various sources, such as gasification of the undigested residue from the fermentation. The ketone hydrogenation follows:

$$RCOR' + H_2 \rightarrow RCHOHR'$$

In conclusion, the MixAlco process is a robust biomass conversion process. It adapts to a wide variety of biomass feedstocks. Because neither expensive enzymes nor

sterilization is required, it is a superb alternative to traditional biomass conversion technologies, such as SSF technology.

1.3.2 Recent advances in the MixAlco process

Recently, the MixAlco process has undergone continuous improvements and achieved several breakthroughs. The improvements are focused on the pretreatment and fermentation sections. Long-term lime treatment with air purged has proven to be an efficient pretreatment method for delignification. The use of marine inocula (i.e., microorganisms from Galveston Island, TX) and countercurrent operations allows higher product concentrations and higher biomass conversions.

Lime ($Ca(OH)_2$) pretreatment has traditionally been used in the MixAlco process, because it is relatively inexpensive, safe to handle, and easy to recover (Holtzapple et al. 1999). Even better, Kim found that lime treatment of corn stover with air purging at mild temperature (i.e., 40–55°C) for 4–6 weeks removed 50% of lignin and all of the acetyl groups (Kim and Holtzapple 2005; Kim 2004). This long-term lime treatment combined with air purging opened a new window for the MixAlco process. Cesar Granda (2004) reported a similar trend for sugarcane bagasse. Lime treatment with air purging significantly enhanced the delignification of sugarcane bagasse compared with lime treatment without air purging. Without air purging, lignin removed from sugarcane bagasse treated with lime only was 20–30%. In contrast, with air purging, lignin removal increased significantly to over 70% at 57°C after 150 days.

The selection of the inoculum source is an important consideration in the anaerobic fermentation. Inoculation of a fermentation system provides the species of microorganisms to the fermentation. The ability of microorganisms to adapt to the new environment determines the final production, yield, and stability of the fermentation process. Extensive research on anaerobic fermentations buffered by calcium carbonate ($CaCO_3$) showed that a marine inoculum was a better inoculum source compared with a

terrestrial inoculum source (Agbogbo 2005; Aiello Mazzarri 2002; Thanakoses 2002). Aiello Mazzarri (2002) compared the fermentation performance of a marine inoculum source with that of a terrestrial inoculum source and concluded that the anaerobic fermentation inoculated from marine inoculum achieved 30% higher total carboxylic acids at 40°C (mesophilic condition). The better performance of marine inoculum source was hypothesized to relate to more "robust" microorganisms that were adapted to the high salt concentration (3.5% salinity) in marine environments.

Countercurrent fermentation is a great improvement to the MixAlco process. High conversions and high product concentrations in the fermentation are possible by using countercurrent operation (Ross and Holtzapple 2001). Countercurrent fermentation allows the least reactive biomass to contact the lowest carboxylic acid concentration, which in batch fermentations could not be digested because of carboxylic acid accumulation. Compared to batch fermentations, this countercurrent arrangement reduces the inhibitory effect from the accumulation of product carboxylate salts by adding fresh liquid to the most digested biomass and continuously removing product from the fermentation system.

In summary, lime treatment, calcium carbonate buffer, marine inocula, and countercurrent fermentation are the key pretreatment and fermentation conditions used in the pilot plant scale. Although economic analysis of the MixAlco process shows these conditions are competitive with other lignocellulosic biomass conversion technologies, more research on the MixAlco process is necessary to make the MixAlco process cost competitive with fossil fuels at traditional prices.

1.4 Project description

The MixAlco process is a good alternative lignocellulosic biomass conversion technology, especially because expensive enzymes are not required. It is well developed and is nearing commercial realization. A MixAlco pilot plant is on operating in College Station, TX.

The study in this dissertation aims to improve the MixAlco process for high ethanol production, due to the growing interest and demand for lignocellulose-based liquid fuels (e.g., ethanol). The direct goal is to achieve high carboxylic acid concentrations, yields, and productivities in fermentations. High percentages of acetic acid are preferred for the biomass-ethanol pathway in the MixAlco process. The ultimate objective is to find the optimum laboratory pretreatment and fermentation conditions and provide some valuable information for future pilot plant scale-up.

This dissertation focuses on pretreatment and fermentation, two major steps in the MixAlco process. The following is a list of detailed objectives performed to meet the main goal:

i) To compare ammonium bicarbonate (NH_4HCO_3), a new buffer system for the MixAlco process, with the previously used calcium carbonate ($CaCO_3$) at 55°C (thermophilic conditions). Lime-treated sugarcane bagasse and office paper, two different substrates, will be evaluated in batch fermentations.

ii) To evaluate effects of both buffer (ammonium bicarbonate and calcium carbonate) on long-term countercurrent fermentations. Lime-treated sugarcane bagasse will be used as substrate in long-term fermentations. The Continuum Particle Distribution Model (CPDM) will be used to model the countercurrent fermentation data and predict the optimum fermentation conditions.

iii) To check the effects of residual calcium salts from the lime treatment of the biomass on the anaerobic fermentation. A hydrogen chloride (HCl) solution will be used to remove the residual calcium from the lime-treated biomass. It will be repeatedly washed with distilled water to ensure clearing of the residual calcium salts as much as possible. The residual calcium ion will be measured in the biomass. The fermentation performance of this specially treated bagasse will be compared with bagasse neutralized by carbon dioxide.

iv) To analyze the effects of biomass pretreatment on the fermentation performance. Hot-lime-water, aqueous ammonia, and air-lime treatments will be compared in both the batch fermentations and the countercurrent fermentations. CPDM will be used to model the countercurrent fermentation data and predict the optimum fermentation conditions.

v) To examine the effect of different inoculum sources on the anaerobic fermentation in the MixAlco process. This study will verify our assumption that the higher salt concentrations in the Great Salt Lake, UT forces the microorganisms to be more “robust” in the MixAlco fermentations.

vi) To study the effect of temperature on anaerobic fermentation performance and obtain some conceptual understanding in the temperature effect. Thermophilic (55°C) and mesophilic (40°C) conditions will be compared for 80% hot-lime-water-treated sugarcane bagasse/20% chicken manure.

CHAPTER II
MATERIALS AND METHODS

This chapter provides a simple guide on the general materials and methods deployed in this dissertation. First, biomass feedstock and pretreatments are summarized. The design of a rotary fermentor, fermentation conditions, and fermentation procedures are then discussed. Analytical techniques for gas and liquid product are also described.

2.1 Biomass feedstock

Both sugarcane bagasse and office paper were used as the carbon source for anaerobic fermentations, whereas chicken manure was selected as the nutrient source for anaerobic fermentations.

2.1.1 Sugarcane bagasse

Sugarcane bagasse, one of the most promising lignocellulosic biomass sources, is generated during the milling of sugarcane. Sugarcane bagasse is plentiful in tropical and subtropical regions (e.g., Brazil, Hawaii, and the southern United States); therefore, sugarcane bagasse was selected as the major biomass feedstock in this dissertation.

Sugarcane bagasse was received from the Lower Rio Grande Valley (LRGV), the location of the sugarcane industry in Texas. Fresh sugarcane bagasse was collected, dried, and ground with a Thomas Wiley laboratory mill (Department of Chemical Engineering, Texas A&M University, College Station, TX) equipped with a 10-mm mesh screen. The moisture content of the ground bagasse was measured. Three

treatment methods (i.e., hot-lime-water treatment, air-lime treatment, and ammonia treatment) were used to enhance the digestibility of sugarcane bagasse.

2.1.2 Office paper wastes

Business and institutions generate huge volumes of waste paper. Disposing of discarded reports, memos, letters, and other office paper waste is expensive and increases pressure on landfills. Using office paper waste as the biomass feedstock can reduce disposal costs and even earn revenues.

Office paper wastes were collected from the wastepaper bin in the graduate student computer lab (Department of Chemical Engineering, Texas A&M University, College Station, TX). The collected waste paper was shredded through a conventional 6-inch paper shredder to achieve a homogeneous size. No additional chemical treatments were deployed to paper waste, because paper pulping already chemically treats the paper.

2.1.3 Chicken manure

Animal wastes (e.g., chicken manure) contain large amounts of protein, fiber, and minerals. Utilizing animal wastes not only provides a cheap nutrient source for anaerobic fermentations, but also has significant environmental benefits. Chicken manure was selected as the nutrient source of anaerobic fermentations, and was received from the Poultry Science Center (Texas A&M University, College Station, TX). Chicken manure was dried and stored for future use.

For all the substrates, volatile solids were determined by the Ross (1998) methodology (Appendix G). Dry matter content was determined by drying the samples overnight in a forced-draught oven at 105ºC (NREL Standard Procedure No. 001). Ash content was determined by heating the samples in a muffle furnace at 550ºC for at least 3 h (NREL Standard Procedure No. 002).

2.2 Biomass pretreatment

Paper did not require additional pretreatment because it was previously chemically pretreated during paper pulping. Sugarcane bagasse, the subject lignocellulosic biomass, was chemically pretreated in this study. Three different treatment methods (i.e., hot-lime-water, lime-air, and ammonia) used for sugarcane bagasse are described as follows:

2.2.1 Hot-lime-water treatment

Hot-lime-water treatment (Appendix A) was performed at 100°C for 2 h with loadings of 0.1 g $Ca(OH)_2$/g dry biomass and 10 mL of distilled water/g dry biomass. Carbon dioxide was bubbled through the biomass slurry to neutralize the residual lime until the pH fell below 7.0. In addition, dilute hydrogen chloride solution instead of carbon dioxide could be used as the neutralization agent. Finally, the slurry was dried at 105°C for 2 days.

2.2.2 Air-lime treatment

Air-lime treatment (Appendix B) was performed at 50°C for 8 weeks with loadings of 0.3 g $Ca(OH)_2$/g dry biomass and 10 mL of distilled water/g dry biomass under air purging. Carbon dioxide was bubbled through the biomass slurry to neutralize the residual lime until the pH fell below 7.0. The resulted biomass slurry was dried at 105°C for 2 days.

2.2.3 Aqueous ammonia treatment

Aqueous ammonia treatment (Appendix C) was performed at 55°C for 24 h with loadings of 10 mL 30% ammonia/g dry biomass. The harvested biomass slurry was washed using distilled water until the pH fell below 7.0. Finally, the slurry was dried at 105°C for 2 days.

2.3 Fermentation materials and methods

2.3.1 Substrates

Paper or treated bagasse was used as the carbon source for anaerobic fermentations, whereas chicken manure was used as the nutrient source for anaerobic fermentations. The preferred ratio is 80 wt% biomass/20 wt% chicken manure (Agbogbo 2005; Aiello Mazzarri 2002).

The average moisture content of chicken manure was 0.052 g water/g chicken manure, the average ash content was 0.340 g ash/g chicken manure, and the volatile solid (VS) content was 0.660 g VS/g chicken manure.

2.3.2 Deoxygenated water

The liquid used in all fermentations consisted of deoxygenated distilled water, sodium sulfide, and cysteine hydrochloride, following the preparation method described in Appendix D. Deoxygenated water was prepared by boiling distilled water and flushing nitrogen for 15 minutes after water reached boiling. After cooling the water to room temperature, 0.275 g/L sodium sulfide and 0.275 g/L cysteine hydrochloride were added as oxygen reducer under nitrogen purge condition. Both sodium sulfide and cysteine hydrochloride were used to eliminate possible residual oxygen in the anaerobic water.

2.3.3 Nutrient mixtures

Table 2-1 lists the components and distribution of dry nutrients used in anaerobic fermentations. The dry nutrients were used as a supplementary nutrient source for the microorganisms, in additional to the major nutrient source (e.g., chicken manure) in anaerobic fermentations. The dry nutrient mixture is more expensive than the biomass nutrient source (manure) and should be used as little as possible. It was prepared as described by Aiello Mazzarri (2002).

Table 2-1. Dry nutrients mixture.

Component	Amount (g/100 g of mixture)
K_2HPO_4	16.3
KH_2PO_4	16.3
NH_2SO_4	16.3
NaCl	32.6
$MgSO_4$ $7H_2O$	6.8
$CaCl_2$ $2H_2O$	4.4
HEPES (N-2-Hydrocyethyl piperazine-N'-2 ethanesulfonate)	0.86
Hemin	0.71
Nicotinamide	0.71
p-Aminobenzoic acid	0.71
Ca-panyothenate	0.71
Folic acid	0.35
Pyrixodal	0.35
Riboflavin	0.35
Thiamin	0.35
Cyanocobalamin	0.14
Biotin	0.14
EDTA	0.35
$FeSO_4$ $7H_2O$	0.14
$MnCl_2$	0.14
H_3BO_3	0.021
$CoCl_2$	0.014
$ZnSO_4$ $7H_2O$	0.007
$NaMoO_4$ $7H_2O$	0.0021
$NiCl_2$	0.0014
$CuCl_2$	0.0007

2.3.4 Inoculum source

Two inoculum sources were selected. Sediment from the seashore of Galveston Island (Galveston, TX) was used as the marine inoculum source. The sediment samples were taken from half-meter deep holes, and stored in 1-L centrifuge bottle filled with anaerobic liquid medium (i.e., deoxygenated water). In addition, sediment from the lakeside of the Great Salt Lake (Salt lake city, UT) was used as the lake inoculum source (Chapter VI).

2.3.5 Methanogen inhibitor

Methanogens should be inhibited to achieve higher carboxylic acid concentration in the fermentation broth, because methane is inexpensive and undesired in the MixAlco process. Iodoform (CHI_3) solution of 20 g iodoform/L ethanol was selected as the methanogen inhibitor in all fermentations, if not otherwise noted. Due to light and air sensitivity, the solution was kept in amber-colored glass bottles and capped immediately after use.

2.3.6 pH Buffer

Ammonium bicarbonate (NH_4HCO_3) or calcium carbonate ($CaCO_3$) was used as pH buffers. A pH of 5.8–6.2 resulted from calcium carbonate buffer, whereas a pH of 6.97–7.03 resulted from ammonium bicarbonate buffer. Urea was also added in calcium carbonate buffered fermentations provided the pH was below 6.0. No urea was required for ammonium bicarbonate buffered fermentations.

The pH was measured and monitored using an ORION portable full-featured pH/temperature meter (Model# 230A). The included Triode™ 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.

2.3.7 Temperature

Most anaerobic fermentations were operated under thermophilic conditions (e.g., 55ºC). Mesophilic conditions (e.g., 40ºC) were also used in Chapter VI. The fermentation temperature was controlled by the incubator temperature.

2.3.8 Fermentor

Rotary fermentors were selected in both batch fermentations and countercurrent fermentations. Figures 2-1 and 2-2 show the rotary fermentor that holds and mixes high-solid biomass slurries. Rotary fermentors were made from Beckman 1-L polypropylene centrifuge bottles (98 × 169 mm, Nalgene brand NNI 3120-1010). The bottle tops were sealed with an 11-inch rubber stopper with a hole drilled in the middle. A glass tube was inserted through the hole and capped with a rubber septum for gas sampling and release. Two 0.25-inch-diameter stainless steel tubes with welded ends were also inserted into holes in the stopper. Both tubes were used as stir bars to mix the biomass slurry inside the fermentors.

Frequent venting gas from the fermentors was necessary to prevent fermentor breakage or explosions, because the maximum pressure limit of the fermentors is 2 atm. The rubber septum was replaced once there was a visible hole due to frequent gas venting.

The rotary fermentors were placed in a Wheaton Modular Cell Production Roller Apparatus (Figure 2-3) located in an incubator consisting of rollers and rotating horizontally at 2 rpm.

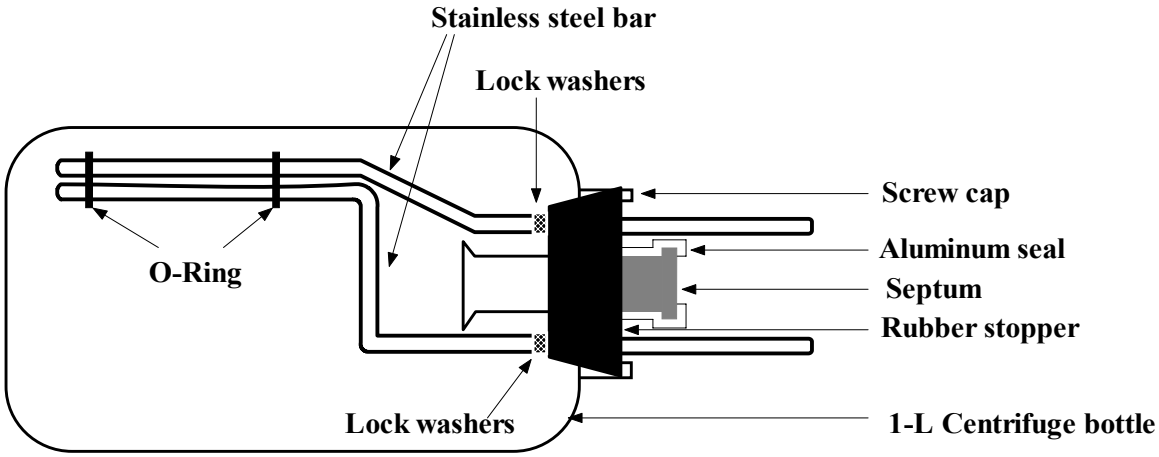


Figure 2-1. Design of rotary fermentor.

Figure 2-2. Photograph of rotary fermentors.

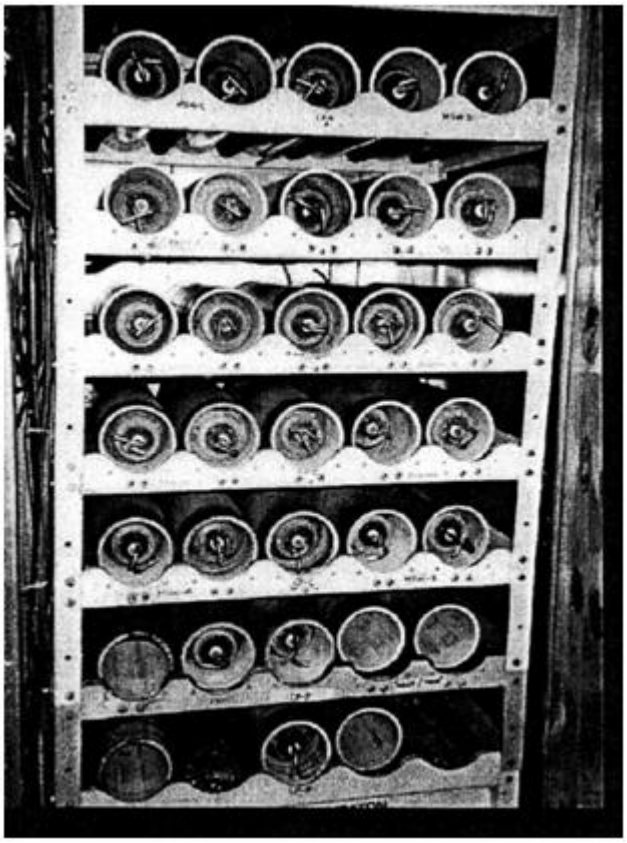


Figure 2-3. Photograph of the fermentation incubator.

2.3.9 Fermentation procedure

Batch experiments

In batch operation, no additional liquid nor solids were added to the fermentation system after the initial charge. Batch experiments were initiated by adding the desired substrates, nutrients, inocula source, and desired pH buffer to the liquid medium in a 1-L rotary fermentor (Figure 2-1). The selected pH buffers were calcium carbonate ($CaCO_3$) or ammonium bicarbonate (NH_4HCO_3). During the preparation process, the fermentors were flushed with nitrogen from a high-pressure liquid nitrogen cylinder to ensure an anaerobic environment for the fermentation. The fermentors were rotated horizontally at

2 rpm in a Wheaton Modular Cell Production Roller Apparatus located in the self-constructed incubator. Batch fermentations were operated under thermophilic conditions (e.g., 55ºC) or mesophilic conditions (e.g., 40ºC).

Countercurrent experiments

In countercurrent operation, the liquid and solids flow in opposite directions in a four-fermentor train. Rotary fermentors were used. Countercurrent fermentations were initiated as batch fermentations until the culture was established (e.g., 7–10 days). The liquid and solids transfer were operated every two days. The liquid produced in one reactor was fed to the next reactor upstream, and the solids from a reactor were moved to the next reactor downstream as described in Figure 2-4. At each transfer session, the fermentors were taken from the incubator and the produced gas was released and measured. The fermentors were opened under nitrogen purging, capped with a centrifuge bottle cap, and centrifuged for 25 min to separate the solids and the liquid. A 3-mL sample of the liquid from Fermentor 1 (F1) was taken for carboxylic acid analysis, and the rest was decanted into a collection bottle for later VS analysis. Solids from Fermentor 4 (F4) were collected in a centrifuge bottle for VS analysis. Fresh biomass was added to F1, and fresh liquid medium was added to F4. The entire transfer process was made under continuous nitrogen purge. A constant wet cake of predetermined weight was maintained in each fermentor to achieve steady-state conditions. Once the

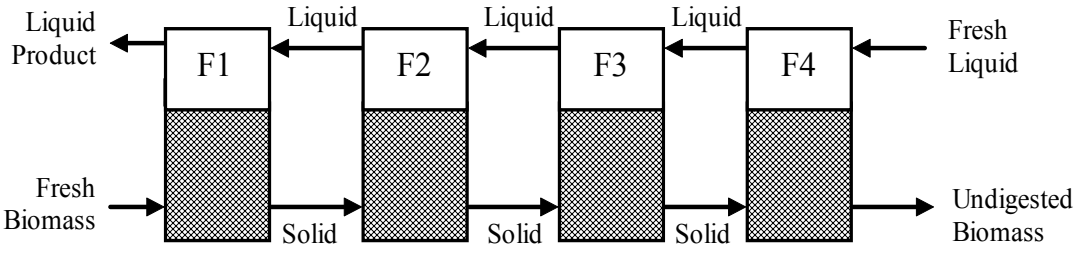


Figure 2-4. Flow diagram of a typical countercurrent fermentation process.

transfer was completed, the fermentors were closed and placed back to the incubator. Steady-state conditions were evidenced when a consistent acid concentration was produced for at least 2 weeks in a row.

2.4 Mass balance of fermentation system

Mass balances were performed in the countercurrent fermentations and the fixed-bed fermentations. Biomass is composed of volatile solids (i.e., VS) and ash. Most of the volatile solids are reactive except lignin, whereas the ash content is nonreactive. Figure 2-5 shows that a fermentation process converts part of the VS into gas and liquid products, with some solids remaining undigested.

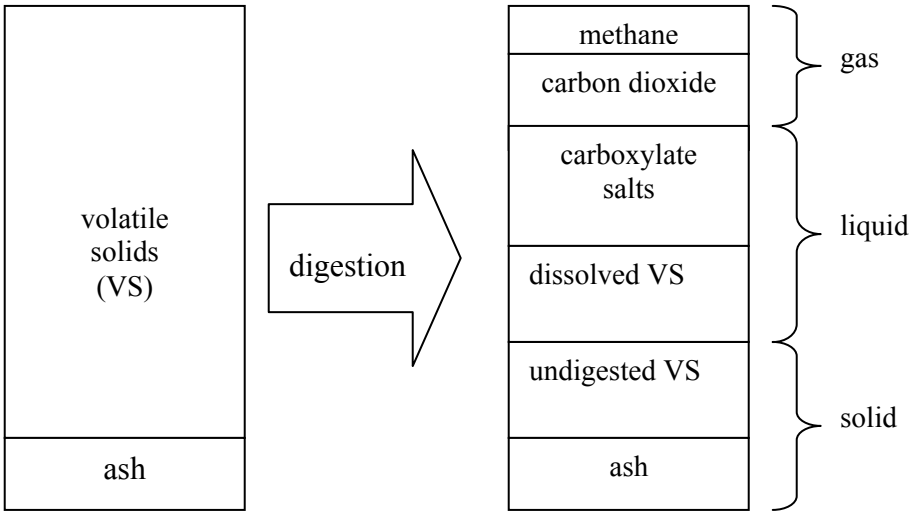


Figure 2-5. Biomass digestion.

For all the countercurrent fermentation experiments, a complete mass balance was obtained on the entire train over a steady-state period. The mass balance closure represents the difference between the mass entering and the mass exiting the

fermentation system. In theory, the mass balance closure should be 100%. Deviations from the expected closure value are due to unavoidable errors in the transfer or measurement process. The mass balance equations are defined as following:

$$\text{VS in} + \text{water of hydrolysis} = \text{undigested VS} + \text{dissolved VS} + \text{carboxylic acids produced} + \text{biotic } CO_2 + CH_4 \quad (2\text{-}1)$$

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

$$\text{VS in} + \text{water of hydrolysis} = \text{VS out} \quad (2\text{-}3)$$

To calculate the water of hydrolysis, Ross (1998) assumed that the biomass could be represented as cellulose, which has a monomer weight of 162 g/mole. When cellulose is hydrolyzed, it gains a molecule of water per monomer; therefore, the water of hydrolysis is calculated as

$$\text{water of hydrolysis} = \text{VS digested} \times \frac{18}{162} \quad (2\text{-}4)$$

Mass balance closure on the entire system was calculated over the steady-state period.

The mass balance closure was calculated as:

$$\text{Closure} = \frac{\text{Mass (out)}}{\text{Mass(in)} + \text{Water of hydrolysis}} \quad (2\text{-}5)$$

$$= \frac{\text{Undigested VS} + \text{Dissolved VS} + \text{Carboxylic Acids} + \text{Biotic } CO_2 + CH_4}{\text{VS(in)} + \text{Water of hydrolysis}} \quad (2\text{-}6)$$

2.5 Definition of terms

2.5.1 Fermentation operating parameters

The operational parameters of the countercurrent fermentations are liquid residence time and volatile solids loading rate.

The liquid residence time determines how long the liquid remains in the system, and also affects the final product concentration. Long liquid residence times allow high product concentrations whereas shorter liquid residence times allow lower product concentrations (Holtzapple et al., 1999). Liquid residence time is calculated as

$$\text{liquid residence time (LRT)} = \frac{\text{TLV}}{Q} \tag{2-7}$$

where,

Q = flowrate of liquid out of the fermentor set (L/d)

TLV = total liquid volume, calculated as

$$\text{Total liquid volume (TLV)} = \sum_i (\overline{K_i} \cdot w + \overline{F_i}) \tag{2-8}$$

where,

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

w = average 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)

The volatile solids loading rate represents the time during which the reactive biomass is added to the system, and is calculated as

$$\text{Volatile solids loading rate (VSLR)} = \frac{\text{VS fed/day}}{\text{TLV}} \tag{2-9}$$

A low VSLR increases the solid residence time, a measurement of how long the solids remain in the fermentation system. Longer solid residence times increase the digestion, and therefore improve product yields. For submerged fermentations, the volume is determined by the LRT and the ratio of solids to liquid. With a high LRT, the cost of the process increases because large capacity volumes are required for the fermentors (Holtzapple et al., 1999).

2.5.2 Fermentation performance parameters

In this dissertation, the following terms are used to evaluate the fermentation performance:

$$\text{conversion} = \frac{\text{VS digested}}{\text{VS fed}} \tag{2-10}$$

$$\text{yield} = \frac{\text{total carboxylic acids produced}}{\text{VS fed}} \tag{2-11}$$

$$\text{total acid selectivity} = \frac{\text{total carboxylic acids produced}}{\text{VS digested}} \tag{2-12}$$

$$\text{total acid productivity} = \frac{\text{total carboxylic acids produced}}{\text{L liquid in all reactors} \times \text{time}} \tag{2-13}$$

2.6 Analytical methods

As mentioned in Section 2.4, gases (e.g., carbon dioxide and methane) accumulate during anaerobic fermentations. Frequently measuring and releasing the accumulated gas avoids possible fermentor explosion.

2.6.1 Gas volume measurement

The volume of produced gas was measured by displacing water in a self-constructed inverted glass graduated cylinder apparatus (Figures 2-6 and 2-7) that was filled with 300 g/L $CaCl_2$ solution. Calcium chloride was used to minimize microbial growth in the water tank, and reduce possible water evaporation. Furthermore, calcium chloride solution prevents CO_2 adsorption, because it has acidic pH (i.e., around 5.6).

To ensure accurate measurements, the reactors were cooled to room temperature before measuring the gas volume. The laboratory equipment allowed four gas volumes to be measured at the same time. 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 was equal to the pressure in the headspace of the cylinder. The recorded water displaced length (L) was converted into produced gas volume (V) using the following equation: $V(\mathrm{mL}) = 19.6 \times L\,(\mathrm{cm})$

2.6.2 Gas content measurement

A gas chromatograph (Agilent 6890 series, Agilent Technologies, Palo Alto, California) equipped with a thermal conductivity detector (TCD) was used to determine the methane and carbon dioxide composition of the fermentation gas. Gas samples were taken directly through the middle rubber stopper of the rotary fermentor using a 5-mL syringe. A standard gas mixture of carbon dioxide (29.99 moL%), methane (10.06 moL%) and the balance nitrogen was routinely used to calibrate the Agilent 6890 gas chromatograph.

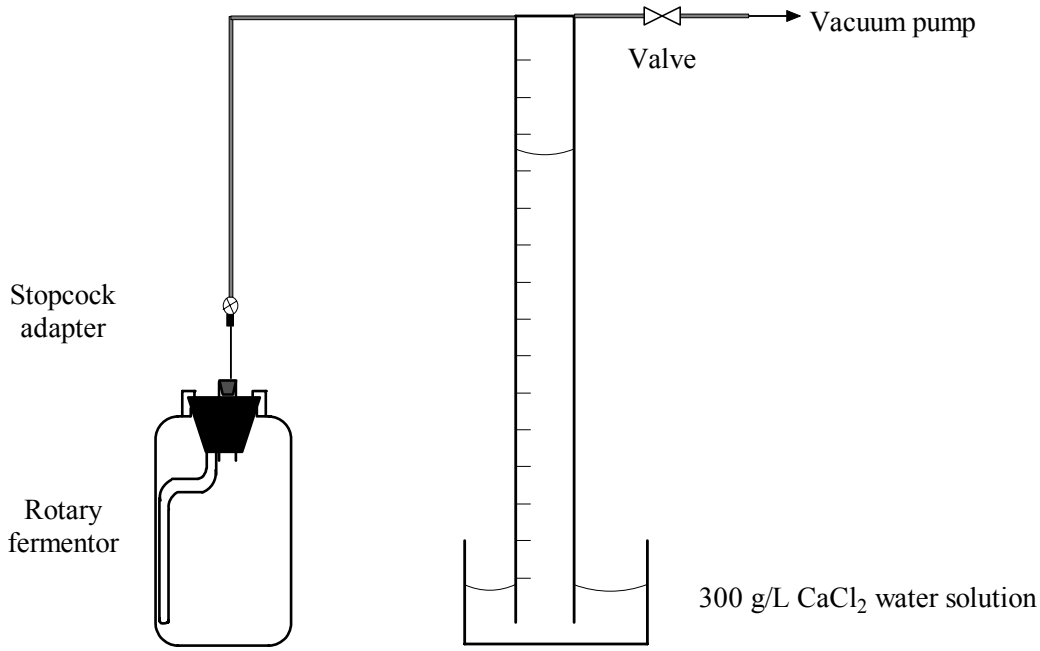


Figure 2-6. Diagram of the water displacement device used to measure gas volume produced from anaerobic fermentations.

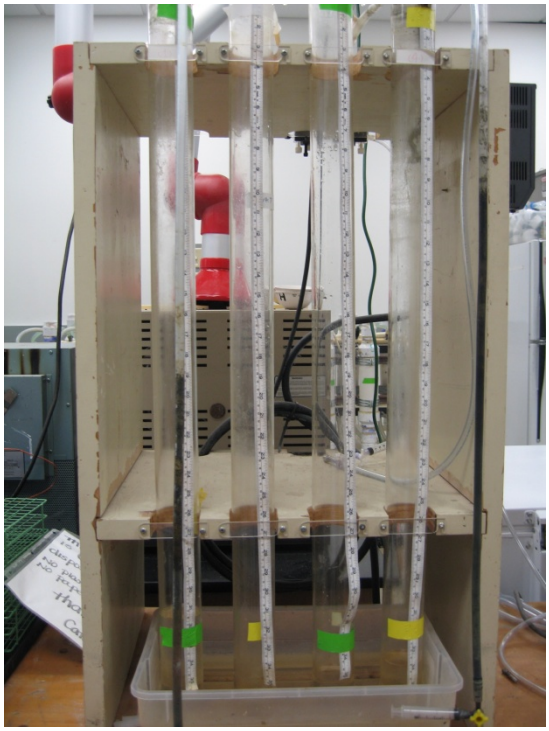


Figure 2-7. Photograph of the water displacement device used to measure gas volume produced from anaerobic fermentations.

2.6.3 Carboxylic acids concentration in liquid samples

A liquid sample of approximately 3 mL was taken from the fermentor. The sample was analyzed immediately or stored in the freezer for future analyze. If frozen, the samples were melted and well mixed before analysis.

Liquid samples were analyzed to measure concentrations of total carboxylic acids using an Agilent 6890 series gas chromatograph (Agilent Technologies, Palo Alto, California) equipped with a flame ionization detector (FID) and a 7683 series injector. Liquid samples were mixed with 1.162 g/L of internal standard solution (4-methyl-n-valeric acid) and acidified with 3-M phosphoric acid. For calibration, a standard carboxylic acids mix (Matreya Inc., catalog #1075) was injected prior to injecting the samples. Acid analysis was performed using an Agilent 6890 gas chromatograph with capillary column (J&W Scientific, model DB-FFAP). It was operated with a flame ionization detector (FID) and an Agilent 7683 Series Injector. The oven temperature in the GC increased from 50^{o}C to 200^{o}C at 20^{o}C/min and was held an additional 1 min at 200^{o}C. More details of liquid samples preparation and analysis are described in Appendix E.

2.6.4 Volatile solid determination

During each transfer schedule, liquid from Fermentor 1 and solids from Fermentor 4 were collected and stored in the freezer for future analysis. The liquid collected from Fermentor 1 after each transfer was analyzed for volatile solids. The solids collected from Fermentor 4 were analyzed for undigested volatile solids. The volatile solid (VS) content of a solid sample was determined by first drying at 105ºC in an oven and then ashing at 575ºC in a furnace for another 3 hours. The VS weight was calculated as the difference between the dry weight and the ash weight. The VS of the liquid samples was determined by adding lime ($Ca(OH)_2$) prior to drying to ensure that the carboxylic acids would not volatilize and alter the measurement.

2.7 CPDM method

The CPDM model was used to predict the countercurrent fermentation using data collected from batch fermentations. CPDM principles are detailed in Chapter VII. Five batch experiments were run simultaneously with different initial substrate concentrations of 40, 70, 100, and 100+ g substrate/L liquid. The 100 and 100+ fermentors had the same initial substrate concentration, but the 100+ fermentor contained a medium with a mixture of carboxylate salts (e.g., 70 wt% calcium acetate, 20 wt% calcium propionate, and 10 wt% calcium butyrate for calcium carbonate buffer) in a concentration of approximately 20 g of carboxylic acids/L liquid. The inoculum for the batch fermentors was taken from countercurrent fermentations operating with the same substrate. Iodoform was added daily to inhibit methane production. Daily samples of the liquid were taken from each fermentor. The amount of produced carboxylic acid measured by gas chromatography was converted to acetic acid equivalents (Aceq). The specific reaction rate as a function of acid concentration (Aceq) and substrate conversion (x) were expressed in Equation 2-14.

$$\hat{r}_{pred} = \frac{e(1-x)^{f}}{1+g(\phi \bullet Aceq)^{h}} \tag{2-14}$$

Nonlinear regression (*SYSTAT SIGMAPLOT 10.0*) was used to determine the parameters e, f, g, and h. The $(1 - x)$ term in the numerator is the *conversion penalty function* described by South and Lynd (1994). The parameter ϕ represents the ratio of moles of acid to moles of acetic acid equivalents.

A self-coded *MatLAB* program based on the CPDM model was used to predict the Aceq and conversion for the countercurrent fermentation at various volatile solid loading rates (VSLR) and liquid residence times (LRT). Furthermore, a "map" could be drawn to show the dependence of the substrate conversion and product concentration for various VSLR and LRT by another self-coded *MatLAB* program. The experimental data collected from the countercurrent fermentation were used to validate the model prediction.

CHAPTER III

A PRELIMINARY COMPARISON OF THERMOPHILIC FERMENTATIONS USING AMMONIUM BICARBONATE AND CALCIUM CARBONATE AS A BUFFER

The objectives of this chapter follow:

a) To determine the feasibility of ammonium bicarbonate (NH_4HCO_3) used as a pH buffer for anaerobic fermentations in the MixAlco process.

b) To compare effects of ammonium bicarbonate (new buffer) and calcium carbonate (old buffer) on anaerobic fermentations and obtain some preliminary result of both buffers based on their fermentation performance (e.g., product concentration and product distribution).

c) To check responses of different biomass feedstocks to both buffers, ammonium bicarbonate and calcium carbonate. Office paper and hot-lime-water-treated sugarcane bagasse are the selected fermentation substrates.

d) To evaluate effects of buffer addition patterns on fermentation performance. Both step-wise addition (e.g., 2 g buffer/4 days) and batch addition (e.g., 4 g buffer in total) will be used.

3.1 Introduction

Anaerobic fermentation is a major operation in the MixAlco process. After the biomass is pretreated to enhance digestibility, it is inoculated with mixed culture of anaerobic microorganisms. Maintaining a stable pH is vital for the growth of anaerobic microorganisms (Joseph F. Malina et al. 1992). During fermentation in the MixAlco process, the biomass feedstock is digested by anaerobic microorganisms, producing carboxylic acids (e.g., acetic acids, propionate acids, and butyric acids) (Holtzapple et al. 1996; Holtzapple et al. 1997). If no pH control is employed, the produced carboxylic acids will lower the pH in the fermentation broth. Consequently, the microorganisms will become inhibited due to the low pH.

pH buffers are chemical agents used in the MixAlco process to maintain a desired pH range and counteract the effects of carboxylic acids produced during fermentations. A buffer, as defined by Van Slyke (1992), is a substance which by its presence in the solution increases the amount of acid or alkali that must be added to cause unit change in pH. In a word, buffers can resist change in hydronium ion (and consequent pH) upon addition of small amounts of acid or base. Buffers are a mixture of a weak acid with its conjugate base or a weak base with its conjugate acid. Table 3-1 lists some important biological buffers, such as sodium acetate, calcium carbonate, and ammonium bicarbonate.

The pH of a solution is a measure of acidity. The smaller the pH, the more acidic the solution. The pH of a solution depends on the concentration of hydrogen ions (H^+) and is calculated by the following equation:

$$pH = -\log[H^+] \tag{3-1}$$

where $[H^+]$ is the concentration of hydrogen ions in the solution, (mol/L).

Table 3-1. The pKa value and buffer range of some important biological buffers.

buffer	pKa 25°C	effective pH range
Acetate	4.76	3.6-5.6
Ammonium hydroxide	9.25	8.8-9.9
AMP (2-amino-2-methyl-1-propanol)	9.69	8.7-10.4
AMPD (2-amino-2-methyl-1,3-propanediol)	8.80	7.8-9.7
BES	7.09	6.4-7.8
BICINE	8.26	7.6-9.0
CAPS	10.40	9.7-11.1
CAPSO	9.60	8.9-10.3
carbonate (pK1) (i.e., bicarbonate)	6.35	6.0-8.0
carbonate (pK2)	10.33	9.5-11.1
CHES	9.50	8.6-10.0
citrate (pK1)	3.13	2.2-6.5
citrate (pK2)	4.76	3.0-6.2
citrate (pK3)	6.40	5.5-7.2
DIPSO	7.52	7.0-8.2
EPPS, HEPPS	8.00	7.6-8.6
ethanolamine	9.50	6.0-12.0
formate	3.75	3.0-4.5
glycine (pK1)	2.35	2.2-3.6
glycine (pK2)	9.78	8.8-10.6
glycylglycine (pK1)	3.14	2.5-3.8
glycylglycine (pK2)	8.25	7.5-8.9
HEPBS	8.30	7.6-9.0
HEPES	7.48	6.8-8.2
histidine	1.70, 6.04, 9.09	5.5-7.4
hydrazine	8.10	7.5-10.0
imidazole	6.95	6.2-7.8
MES	6.10	5.5-6.7
methylamine	10.66	9.5-11.5
phosphate (pK1)	2.15	1.7-2.9
phosphate (pK2)	7.20	5.8-8.0
phosphate (pK3)	12.33	
POPSO	7.78	7.2-8.5
propionate	4.87	3.8-5.6
pyridine	5.23	4.9-5.9
pyrophosphate	0.91, 2.10, 6.70, 9.32	7.0-9.0
succinate (pK1)	4.21	3.2-5.2
succinate (pK2)	5.64	5.5-6.5

The resistive action of a buffer to pH changes results from the chemical equilibrium between buffer pairs (i.e., the weak acid and its conjugate base or the weak base and its conjugate acid). The pH in a buffered solution is related with the buffer pair and can be calculated by the Henderson-Hasselbalch equation:

$$pH = pK_a + \log\left(\frac{[\text{basic species}]}{[\text{acidic species}]}\right) \tag{3-2}$$

where: pKa is the dissociation constant of the acids.

Figures 3-1 and 3-2 show different responses of the unbuffered solution and buffered solution to acid addition, respectively. This type of pH response, the so-called titration curve, is made by plotting the pH against the volume of acid or base added to a solution (Kirschenbaum et al. 1972). Figure 3-1 shows how the pH in an unbuffered solution responds to strong acid, whereas Figure 3-2 exhibits the pH in a buffered solution with the same addition of acids. In Figure 3-1, the solution started as 25 mL of 1-M alkali solution (e.g., sodium hydroxide). A 1.25-M HCl solution is slowly added to decrease the pH. The pH decreases a very small amount in the initial stages, then there is a steep plunge near the equivalence point. The pH falls from 11.44 (19.9 mL HCl added) to 2.56 (20.1 mL HCl added) when only 0.2 mL HCl is added. The lack of buffer in this solution leads to no "defense" (8.88 pH unit change) to the added acid concentration.

Figure 3-2 shows that a buffered solution behaves differently. When a small amount of acid is added to a buffered solution (e.g., sodium carbonate), the buffer reacts with the introduced H^+ and stabilizes the pH changes. The pH drops from 8.46 (19.9 mL HCl added) to 8.29 (20.1 mL HCl added) when only 0.2 mL HCl is added. The pH change of the buffered solution (0.17 pH unit change) is much less than that of the unbuffered solution (8.88 pH unit changed). In conclusion, buffer plays an important role in stabilizing the pH change compared to an unbuffered solution.

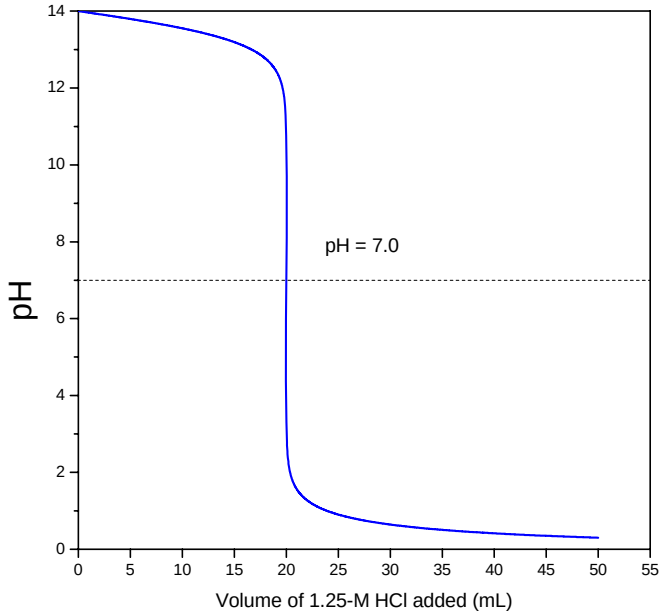


Figure 3-1. A typical titration curve of an unbuffered solution, where 25 mL of 1-M sodium hydroxide (NaOH) solution is titrated by 1.25-M HCl solution.

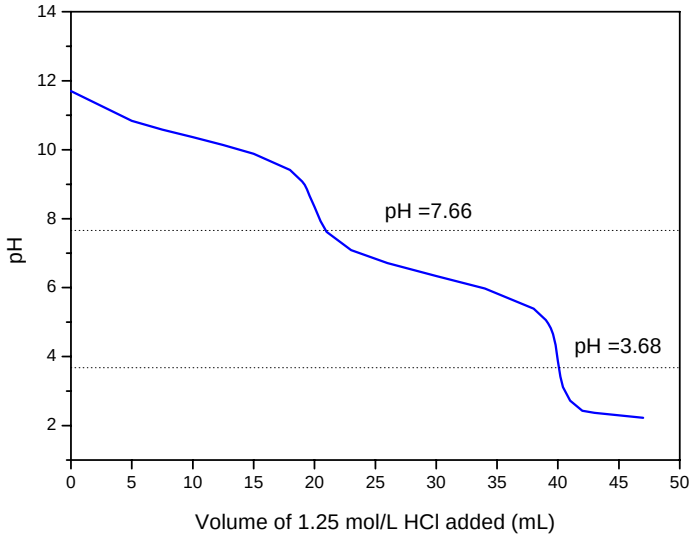


Figure 3-2. A typical titration curve of an unbuffered solution, where 25 mL of 1-M sodium hydroxide (Na_2CO_3) solution is titrated by 1.25-M HCl solution.

The buffering capacity of the buffer system is another factor that must be considered in fermentation design. The higher concentration of buffer, the greater the buffer capacity. In general, the most buffering capacity of the buffer system is available when the concentration of weak acid or base is close to the concentration of the conjugate ion. Under this situation, the term [basic species]/[acidic species] in Equation 3-2 will be nearly equal to 1. For a typical anaerobic fermentation in the MixAlco process, the fermentation system continuously produces carboxylic acids. Even without additional acids/base added to the fermentation system, these produced carboxylic acids will break the chemical equilibrium of the buffer pairs, which leads to an undesired pH range if no buffer is added.

Calcium carbonate ($CaCO_3$) was reported as a successful buffer and has been widely studied in the MixAlco process (Aiello Mazzarri 2002; Chan and Holtzapple 2003; Thanakoses 2002). Calcium carbonate is a good choice because it is cheap and safe to handle. Calcium carbonate consumed in anaerobic fermentations can be recycled and converted to lime which is an effective pretreatment agent used in the MixAlco process. The pH buffering range around 6.0 makes calcium carbonate a natural "methane inhibitor," because many methane-producing microorganisms are inhibited around pH 6.0. The inhibition is not perfect, so an inhibitor, such as iodoform, must be added (Chan and Holtzapple 2003; Thanakoses 2002).

Most microorganisms thrive under neutral conditions (i.e., pH 7.0). Using calcium carbonate to maintain pH around 6.0 discourages the growth of many potentially desirable microorganisms that can convert the biomass into carboxylic acids. Therefore, a new buffer with pH buffer range around 7.0 can be introduced to the MixAlco process. Because methanogens prosper at pH 7.0, it may be necessary to add a methanogen inhibitor, such as iodoform.

Ammonium bicarbonate (NH_4HCO_3) is a good potential buffer candidate. 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). Table 3-2 compares ammonium bicarbonate and calcium carbonate in terms of general chemical and physical properties. Ammonium bicarbonate is desirable because the pH buffer range of bicarbonate salts is near pH 7.0 (Table 3-1). Also, ammonia is an essential nutrient for anaerobic microbes (Katagiri and Nakamura 2002). Total ammonia nitrogen (TAN) concentrations of approximately 200 mg/L are believed to benefit anaerobic fermentations. Amino carboxylate salts provide both a carbon and nitrogen source when used as animal feed. Other benefits of ammonium salts are inhibition of methanogenesis (Kayhanian, 1998; Parkin et al., 1980) and prevention of scale formation in downstream heat exchangers.

In summary, the study in this chapter was undertaken to investigate the feasibility of applying ammonium bicarbonate buffer to maintain a desired pH range for anaerobic fermentations. Ammonium bicarbonate (new fermentation buffer) will be compared with calcium carbonate (old fermentation buffer) in both paper fermentations and sugarcane bagasse fermentations.

Table 3-2. General physical and chemical properties of ammonium bicarbonate and calcium carbonate.

buffer	Ammonium bicarbonate	Calcium carbonate
Formula	NH_4HCO_3	$CaCO_3$
Solubility (saturated aqueous concentration)	high solubility in water, 31.6 wt% at 50°C; 26.8 wt% at 40°C.	very low solubility in water, 6.7×10^{-6} wt% at 25°C
Reactivity with acids	reacts with acids to yield gaseous carbon dioxide (1 moL abiotic CO_2 /moL $[H^+]$) $HCO_3^- + H^+ = H_2O + CO_2$	reacts with acids to yield gaseous carbon dioxide (1/2 moL abiotic CO_2 /moL $[H^+]$) $CO_3^{2-} + 2H^+ = H_2O + CO_2$
Reactivity with alkalis	reacts with alkalis to yield gaseous ammonia	does not react with alkalis
Safety	corrosive to nickel, copper, and many of their alloys no reactive to stainless steel, aluminum, glass, ceramics, rubber, and plastics	safe and no reactive to most of alloys

3.2 Methods and materials

Table 3-3 summarizes the pretreatment and fermentation conditions used in this chapter.

3.2.1 Selection of biomass feedstock

Office paper and sugarcane bagasse were selected as the carbon sources for fermentations in this chapter. Chicken manure was chosen as the main nutrient source to lower the usage of expensive nutrient mixture. The mixture of 80% biomass and 20% raw chicken manure was the initial substrate for all batch fermentations in this chapter.

Office paper was prepared as described in Chapter II. The ground sugarcane bagasse was pretreated by lime ($Ca(OH)_2$) at 100°C for 2 hours followed by carbon dioxide neutralization. The pretreated bagasse was dried in an oven at 105°C. The average volatile solid content for the lime-treated bagasse was 83.8%. The average volatile solid content for the raw chicken manure was 74.4%.

3.2.2 Thermophilic fermentations

In this chapter, batch fermentations were used in a preliminary study. The batch fermentation procedures are detailed in Chapter II (Materials and Methods). The liquid volume in all fermentations was 250 mL. The temperature was maintained around 55°C (thermophilic conditions). The substrate, 20 g of 80% biomass/20% raw chicken manure, was the initial biomass feedstock for batch fermentations. The fermentation configurations are listed in Table 3-3. All of the batch fermentations were started at the same time and operated under identical conditions.

Two different buffers, ammonium bicarbonate and calcium carbonate, were used to adjust pH to the desired range during the fermentation procedure. Both step-wise addition and batch addition of buffer were used.

Table 3-3. Matrix table for buffer comparison.

Operating conditions		**Case**	**Used in this chapter**
Substrate (nutrient source)		Chicken manure	√
Substrate (carbon source)		Bagasse	√
		Paper	√
Bagasse Pretreatment	Chemical	Lime solid, $Ca(OH)_2$	√
		Aqueous ammonia, $NH_3 + H_2O$	
	Temperature	55°C	
		100°C	√
		Room temperature (20–25°C)	
	Time	2 hours	√
		1 day	
		12 days	
		1 month	
	Neutralization	Carbon dioxide, CO_2	√
		Hydrogen chloride, HCl	
		Acetic acid, CH_3COOH	
		D.I. water washing, no chemicals	
	Drying method	105°C Oven (2 d)	√
		Room temperature hood (2 d)	
Fermentation	Temperature	Thermophilic conditions (55°C)	√
		Mesophilic conditions (40°C)	
	Neutralization buffer	Ammonium bicarbonate, NH_4HCO_3	√
		Calcium carbonate, $CaCO_3$	√
	Methane inhibitor	Iodoform	√
	Inoculum source	Original (unadapted) marine inoculum	
		Adapted marine inoculum from previous countercurrent fermentation	√
		Original (unadapted) lake inoculum	

3.3 Results and discussions

3.3.1 Reproducibility of thermophilic fermentations using ammonium bicarbonate as a buffer

In this chapter, the anaerobic fermentation using ammonium bicarbonate was a first try under thermophilic conditions for the MixAlco process. Four batch fermentations were used to check the reproducibility of thermophilic fermentations using ammonium bicarbonate as a buffer. The four fermentations were operated under identical conditions. They were started from 100 g/L substrate concentration with 80% lime-treated bagasse and 20% chicken manure. Ammonium bicarbonate was used to adjust the pH near 7.0 whenever the fermentor was opened to take liquid sample.

Figures 3-3 and 3-4 show the carboxylic acids produced from thermophilic fermentations using ammonium bicarbonate as a buffer. At the beginning of the fermentation (first 7 days), the total carboxylic acid concentration was very similar. The variation became larger as fermentations progressed; however, the t-test with 95% confidence interval indicates that the reported fermentation data were not statistically different from each other. Thus, the ammonium bicarbonate thermophilic fermentation is reproducible. Furthermore, the steadily increased carboxylic acids concentration during fermentation demonstrated that ammonium bicarbonate is a feasible buffer for anaerobic fermentations under thermophilic conditions. The anaerobic microorganisms could adapt to this new buffer and continuously produce carboxylic acids. Therefore, further investigations could be continued for this new buffer (ammonium bicarbonate).

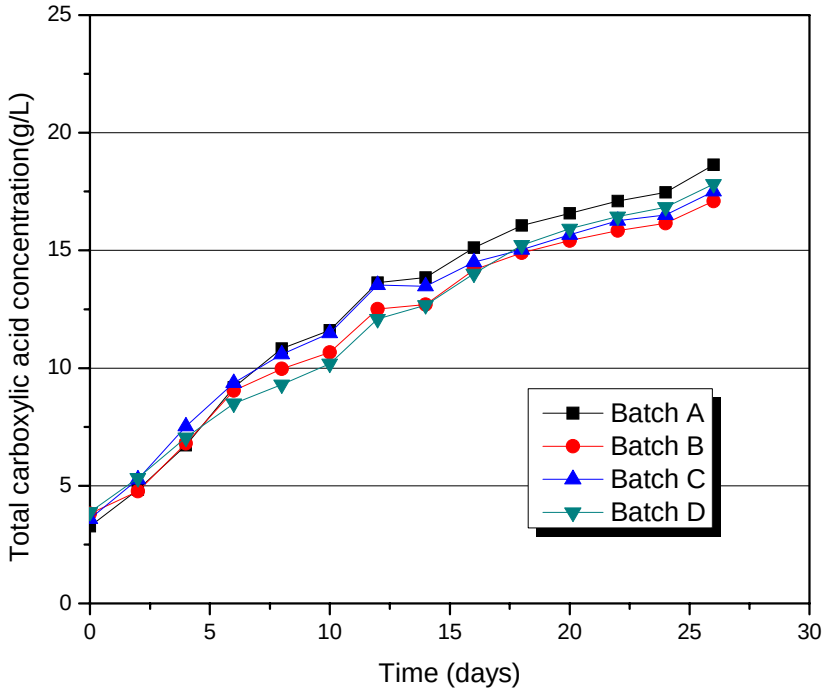


Figure 3-3. The total carboxylic acid concentrations in four identical bagasse fermentations using ammonium bicarbonate as a buffer under thermophilic conditions.

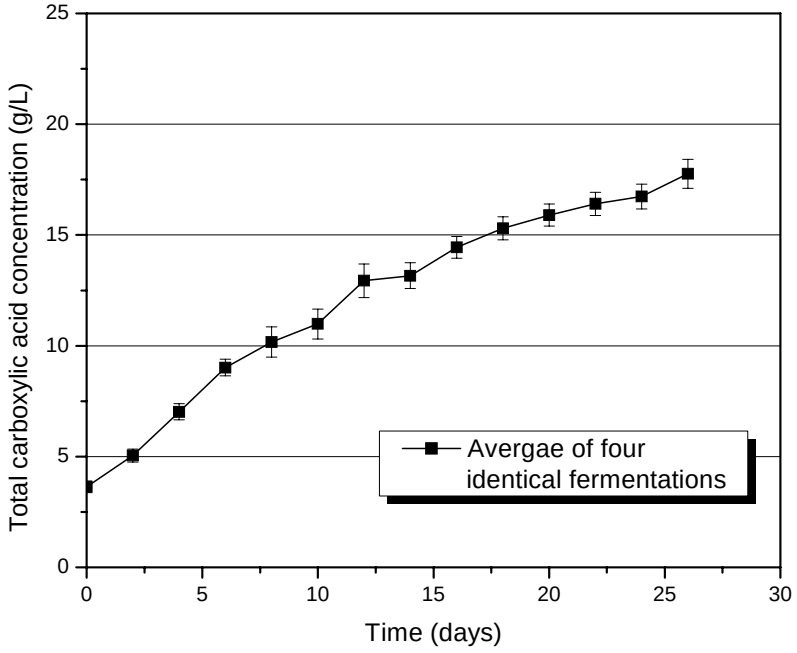


Figure 3-4. The total carboxylic acid changed with time from four identical bagasse fermentations using ammonium bicarbonate as a buffer under thermophilic conditions. Error bar indicates ± 1 standard deviation.

3.3.2 Paper fermentation

As mentioned before, office paper is chemically pretreated in the paper pulping process. Office paper requires no additional chemical pretreatment to enhance digestibility for anaerobic fermentations in the MixAlco process (Aiello Mazzarri 2002). Paper is a desirable biomass substrate in a preliminary comparison between ammonium bicarbonate and calcium carbonate, because the required pretreatment for other biomass substrate may introduce additional salts (e.g., calcium salts from lime pretreatment) to the fermentation broth and may interfere with fermentation performance.

Four paper fermentations (Fermentation P1–P4 in Table 3-4 and Figure 3-5) were established to compare the performance of ammonium bicarbonate and calcium carbonate under thermophilic conditions. Office paper (16 g), raw chicken manure (4 g), urea (0.2 g), nutrients (0.2 g), anaerobic water (230 mL), and inocula (20 mL) were used in each fermentation. Fermentations P1–P3 used ammonium bicarbonate, whereas

Table 3-4. Selected configurations for paper fermentation.

	Buffer	Inoculum source
P1	Ammonium bicarbonate, NH_4HCO_3	20 mL adapted inocula from previous countercurrent bagasse fermentations under thermophilic conditions
P2	Ammonium bicarbonate, NH_4HCO_3	20 mL adapted inocula from previous countercurrent bagasse fermentations under thermophilic conditions
P3	Ammonium bicarbonate, NH_4HCO_3	20 mL adapted inocula from previous batch paper fermentations under mesophilic conditions (Agbogbo 2005)
P4	Calcium carbonate, $CaCO_3$	20 mL adapted inocula from previous countercurrent bagasse fermentations under thermophilic conditions

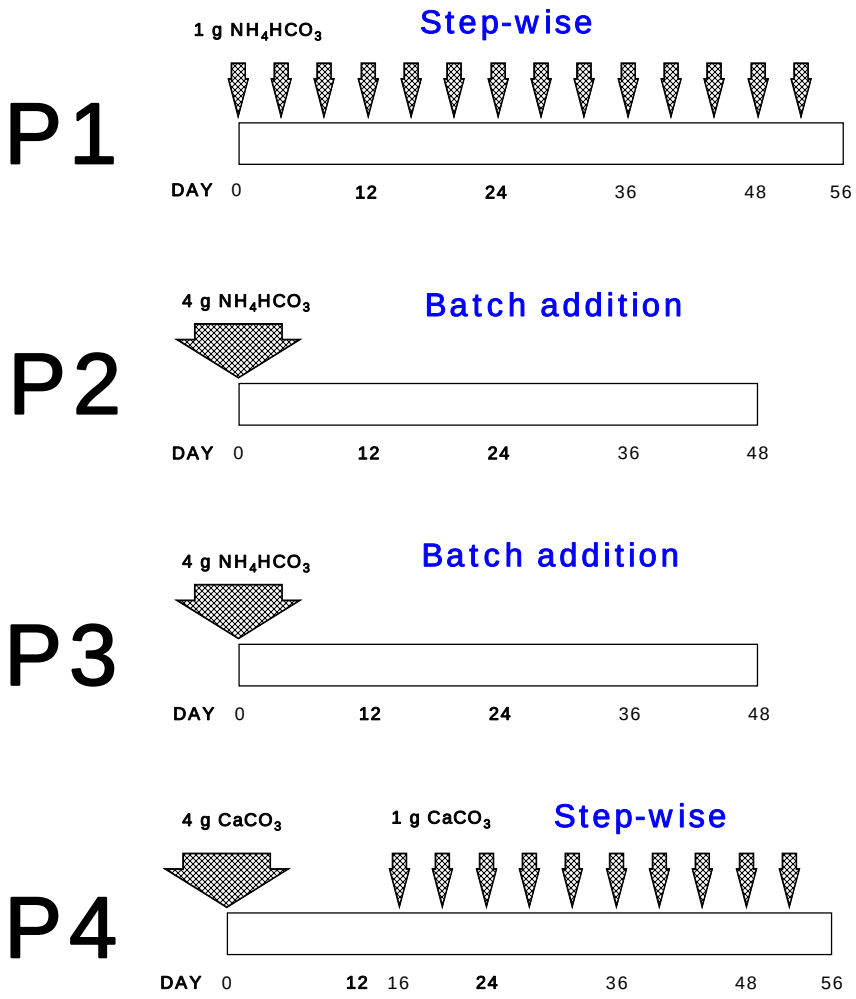


Figure 3-5. Selected buffer addition patterns for paper fermentations under thermophilic conditions (55°C).

Fermentation P4 used calcium carbonate. Iodoform solution (120 μL) was added every two days to inhibit methanogens and 3 mL of liquid was taken as a sample.

Figure 3-6 shows paper fermentation performance and demonstrates that the product concentration will change due to the different pH buffers. In the first week, the anaerobic microorganisms from the inoculum source started to grow. There was not much difference in product concentration for all fermentations using ammonium bicarbonate. However, Fermentation P4 using calcium carbonate had less product concentration during this period. After this period, the fermentation with step-wise addition of ammonium bicarbonate (Fermentation P1) began to exceed all of other fermentations. The product concentration reached 15.0 g/L in 14 days, 22.0 g/L in 20 days, and around 40.0 g/L in 50 days. In contrast, Fermentation P4 (with calcium carbonate) produced 7.0 g/L in 14 days, reached 9.0 g/L in 20 days, and around 22.0 g/L in 50 days. There is a significant product concentration difference between the two buffer systems. For paper substrate, total product concentrations for fermentations using ammonium bicarbonate were nearly double those of fermentation using calcium carbonate.

The relatively low carboxylic acid production from Fermentations P3 and P4 indicate that the chemical property of the buffer is not the only factor that affects fermentation performance. The buffer addition pattern also makes a difference. Fermentations P2, P3, and P4 used identical ammonium bicarbonate as buffer, but with a different addition pattern. The step-wise addition used in Fermentation P1 is a better choice. Therefore, the step-wise addition pattern is preferred for ammonium bicarbonate buffer.

Ammonium bicarbonate buffered fermentation is sensitive to pH. The high initial pH (over 8.0) is bad for anaerobic fermentations using ammonium bicarbonate. If the pH is above 8.0, there is a low product concentration. Microorganisms are inhibited

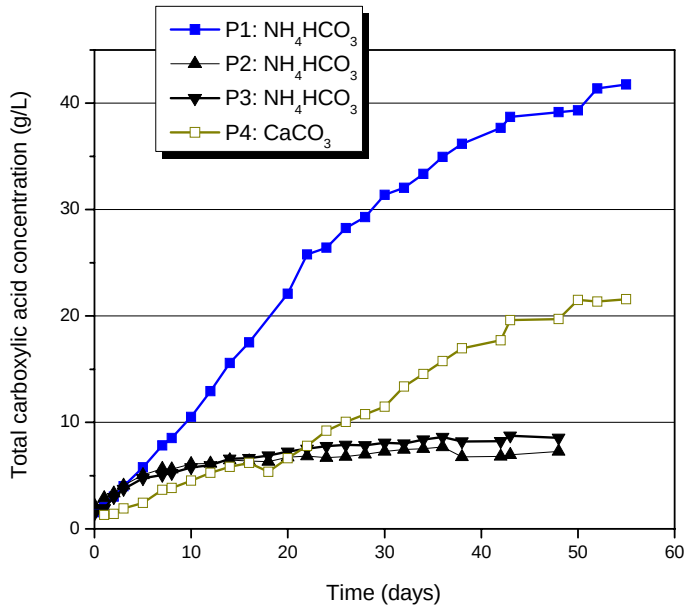


Figure 3-6. The total carboxylic acid changed with time for paper fermentations under thermophilic conditions. Ammonium bicarbonate and calcium carbonate were used as buffer.

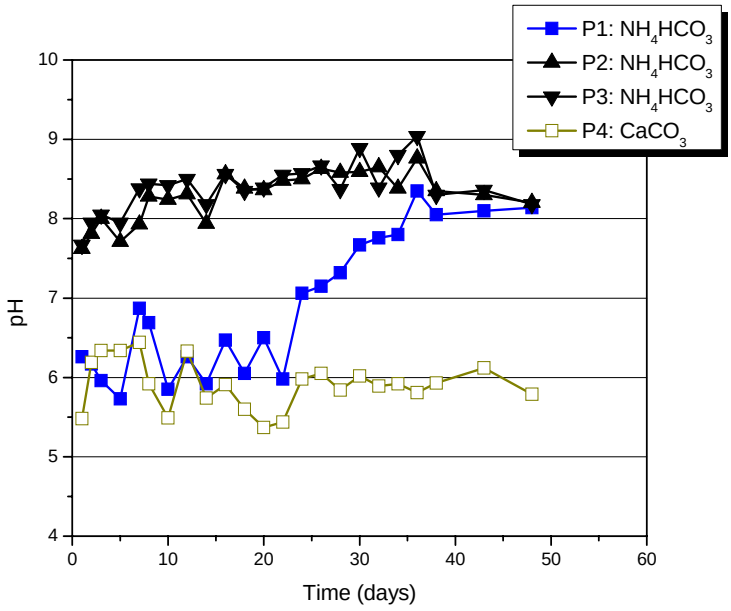


Figure 3-7. pH profiles for paper fermentations under thermophilic conditions. Ammonium bicarbonate and calcium carbonate were used as buffer.

under such high pH conditions. Although Fermentations P3 and P4 used ammonia bicarbonate as Fermentation P2, the pH ranged between 7.8 and 8.2 (Figure 3-7) in the first three weeks was believed to result in a low total product concentration. Due to the weak fermentation performance compared to Fermentation P2, Fermentations P3 and P4 was terminated at week 8. On the other hand, a pH range of 6.5–7.5 seems ideal and preferred for fermentations using ammonium bicarbonate. Better control of ammonium bicarbonate addition must be considered in future studies to maintain a "healthy" pH environment, especially for the first three weeks.

The increased percentage of acetate in the carboxylic acids is an exciting discovery. High acetate content (over 92%) in fermentation broth is possible under thermophilic conditions. Figure 3-8 shows that fermentations using ammonium bicarbonate achieved significantly higher acetate content than fermentations using calcium carbonate. The acetate content using ammonium bicarbonate buffer was about 92% in thermophilic fermentations (e.g., Fermentation P1), whereas the acetate content was around 68% in fermentations using calcium carbonate buffer (Fermentation P4). This value is close to the 65% acetate content for thermophilic fermentations using calcium carbonate in previous research (Chan and Holtzapple 2003).

The high acetate content (over 92%) in the product can be helpful in some situations. As mentioned before, the concentrated carboxylic salts (or acids) from the fermentation broth can be converted to mixed alcohols in the MixAlco process. If ethanol is the desired product, thermophilic fermentations with ammonium bicarbonate buffer would produce 92% of the mixed alcohols as ethanol.

In summary, using ammonium bicarbonate buffer in paper fermentations under thermophilic conditions is feasible and has great advantages over using calcium carbonate buffer by achieving higher total carboxylic acid concentration and higher acetate content. We may safely conclude that ammonium bicarbonate is a better buffer than calcium carbonate for anaerobic fermentations under thermophilic conditions.

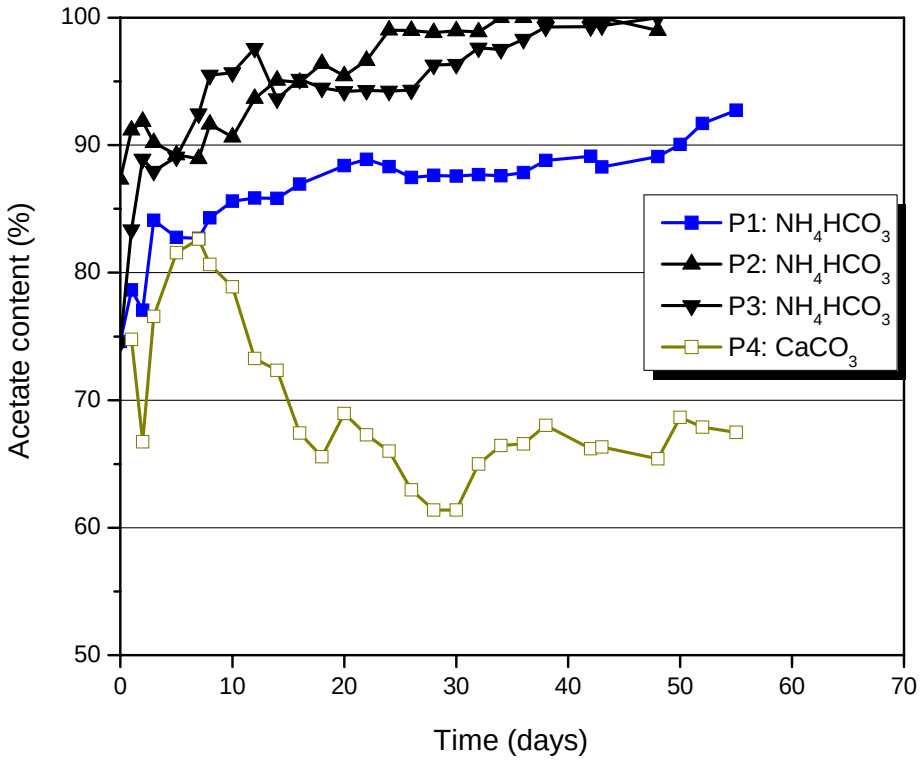


Figure 3-8. Acetate content profile for carboxylic acids produced in paper fermentations under thermophilic conditions. Ammonium bicarbonate and calcium carbonate were used as buffer.

3.3.3 Bagasse fermentation

Sugarcane bagasse, a collected agriculture waste, is a desirable biomass feedstock and was selected as the major biomass feedstock in this dissertation. Lime-pretreated bagasse was used in this section to compare calcium carbonate and ammonium bicarbonate.

Four different fermentation configurations using bagasse (B1–B4 in Table 3-5 and Figure 3-9) were established to compare the performance of ammonium bicarbonate and calcium carbonate under thermophilic conditions. Fermentations B1 and B2 used calcium carbonate buffer, whereas Fermentations B3 and B4 used ammonium bicarbonate buffer. Hot-lime-water-treated sugarcane bagasse (16 g), raw chicken manure (4 g), nutrients (0.2 g), anaerobic water (230 mL), and inocula (20 mL) were used in each fermentation. Urea (0.2 g) was added to Fermentations B1 and B2. The same inocula from the previous countercurrent bagasse fermentations using calcium carbonate buffer was employed in this section. Based on the success of step-wise buffer addition in paper fermentations (Section 3.3.2), both buffers were added using the step-wise addition pattern in this section.

Table 3-5. Selected configurations for hot-lime-water-treated bagasse fermentation.

	Buffer	Inoculum source
B1	Calcium carbonate, $CaCO_3$	20 mL adapted inocula from previous countercurrent bagasse fermentations under thermophilic conditions
B2	Calcium carbonate, $CaCO_3$	
B3	Ammonium bicarbonate, NH_4HCO_3	
B4	Ammonium bicarbonate, NH_4HCO_3	

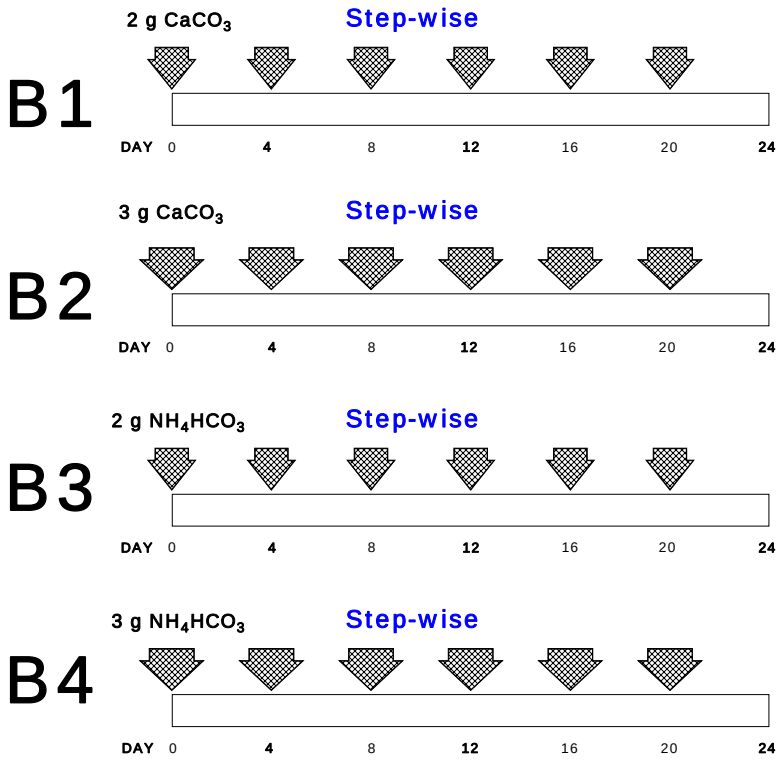


Figure 3-9. Selected buffer addition patterns for hot-lime-water-treated bagasse fermentations under thermophilic conditions (55°C).

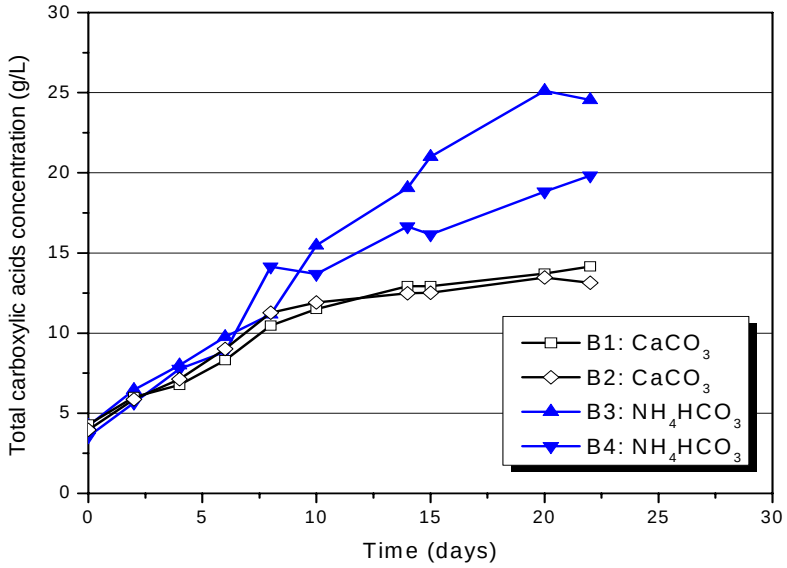


Figure 3-10. Total carboxylic acid concentration changed with time for hot-lime-water-treated bagasse fermentations under thermophilic conditions. Ammonium bicarbonate and calcium carbonate were used as buffer.

Figure 3-10 shows the carboxylic acid concentration of bagasse fermentation under thermophilic conditions, whereas Figure 3-11 shows pH in the fermentation broth. There was not much difference in total carboxylic acids production in the first 6 days between ammonium bicarbonate and calcium carbonate buffers. The microorganism culture was still developing during this period. Once the culture was developed, the total carboxylic acids production began to show differences. Thermophilic fermentations using ammonium bicarbonate buffer obtained higher product concentration. In 22 days, the average of product concentration in ammonium bicarbonate buffered fermentation was around 22.0 g/L. On Day 22, the total product concentration using ammonium bicarbonate was about 50–60% higher than using calcium carbonate, which averaged 14.0 g/L for calcium carbonate buffered fermentations. Again, the higher product concentration shows that ammonium bicarbonate is a better buffer for the anaerobic fermentations.

Figure 3-11 shows that thermophilic fermentations are not sensitive to calcium carbonate addition rate, whereas they are sensitive to ammonium bicarbonate addition rates. There was no significant difference in pH for 2 g/4 days and 3 g/4 days step-wise addition of calcium carbonate. The pH is well maintained around 5.8 for both addition rates of calcium carbonate (Fermentations B1 and B2). In contrast, ammonium bicarbonate addition rates significantly affect fermentation performance. Step-wise buffer addition pattern are preferred for thermophilic fermentations using ammonium bicarbonate. Ammonium bicarbonate addition patterns affect product concentration more than calcium carbonate addition patterns. A step-wise addition of ammonium bicarbonate of 2 g/4 days achieved higher product concentrations than 3 g/4 days step-wise buffer addition. The design of the rotary fermentator makes it impossible to apply feedback-controlled buffer addition, which could automatically add buffer to maintain a desired pH range based on the real-time pH changes in the fermentation broth. In an industrial scale, feedback-controlled buffer addition is possible and should be employed.

Based on the responses from both paper fermentation and bagasse fermentation, ammonium bicarbonate is a better buffer. Further investigations will focus on ammonium bicarbonate buffered fermentations in Chapter IV. Long-term fermentation performance will be used to evaluate the role of ammonium bicarbonate in Chapter VIII.

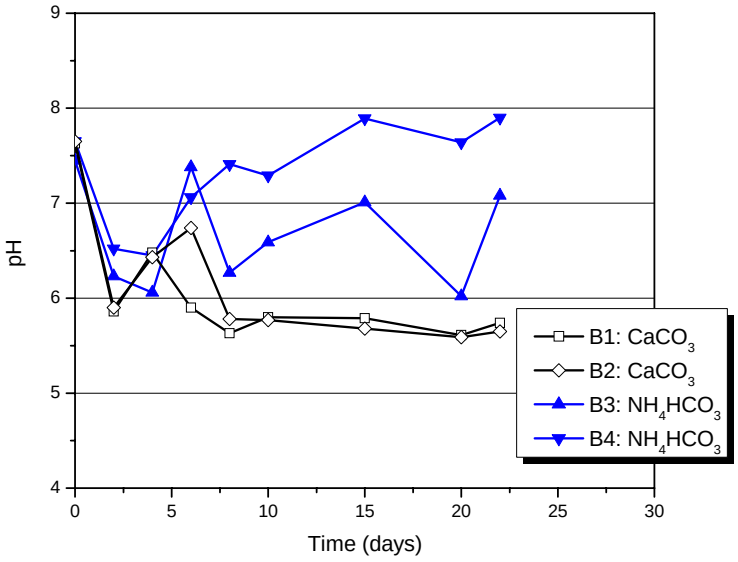


Figure 3-11. pH profiles for hot-lime-water-treated bagasse fermentations under thermophilic conditions. Ammonium bicarbonate and calcium carbonate were used as buffer.

3.4 Conclusions

It has been demonstrated that using ammonium bicarbonate as a buffer is feasible in anaerobic fermentations under thermophilic conditions. Fermentations using ammonium bicarbonate produce more carboxylic acids for both sugarcane bagasse and office paper than fermentations using calcium carbonate. The following conclusions have been made based on batch fermentation performance at 55°C:

1) Ammonium bicarbonate is a better buffer than calcium carbonate under thermophilic conditions. The total product concentrations from paper fermentations using ammonium bicarbonate is almost double that using calcium carbonate, if the pH of ammonium bicarbonate buffered fermentation is maintained around 7.0. There is around 50–60% increase of total carboxylic acid concentration for bagasse fermentations.
2) Acetate content of total carboxylic acids fermented from office paper using ammonium bicarbonate could reach about 92% under thermophilic conditions. This is higher than thermophilic fermentations using calcium carbonate, which were ~70% acetate.
3) Fermentations buffered by ammonium bicarbonate are pH sensitive. If the pH is 8.0 or above, the product concentration is low. The desired pH range should be controlled within 6.5–7.5.
4) If the pH is above 8.0, the acetate content is approximately 95%.
5) Ammonium bicarbonate addition patterns affect product concentration more than calcium carbonate addition patterns. For paper fermentation, 16 g/L ammonium bicarbonate batch addition rate raised the pH and inhibited the microorganisms thus destroying thermophilic fermentation. In contrast, because it is insoluble, 16 g/L calcium carbonate addition rate did not significantly affect the microorganism culture. Step-wise buffer addition is recommended for ammonium bicarbonate buffer.

CHAPTER IV

INVESTIGATION ON ANAEROBIC FERMENTATION USING AMMONIUM BICARBONATE AS A BUFFER

The objectives of this chapter follow:

a) To continue comparing fermentation performance using ammonium bicarbonate and calcium carbonate buffers under controlled pH (around 7.0).

b) To check the role of ammonium bicarbonate in fermentations, and to examine whether ammonium bicarbonate could function as a "methane inhibitor" and fully replace iodoform.

c) To evaluate the feasibility of ammonia pretreatment of biomass used for ammonium bicarbonate buffered fermentations.

d) To find suitable operation parameters for ammonia pretreatment by trial-and-error methods. Long-term treatment (12 days) and short-term treatment (1 day) are examined.

This chapter is a collection of several brainstorming and exploratory investigations of ammonium bicarbonate buffered fermentations. The previous chapter shows that ammonium bicarbonate is a better buffer than calcium carbonate. All of the experiments in this chapter are therefore designed to make full use of ammonium bicarbonate in anaerobic fermentations. Trial-and-error is widely used here. Continuous comparison of ammonium bicarbonate and calcium carbonate was performed under controlled pH, whereas the buffer comparison in Chapter III is based on a batch addition of fixed amount of buffer. This is followed by an investigation into the mechanism of ammonium bicarbonate in fermentations, with main focus on its potential as a "methane inhibitor." The last part of this chapter is dedicated to evaluating the feasibility of ammonia pretreatment prior to ammonium bicarbonate buffered fermentations.

4.1 Continuous comparison of buffers under controlled pH

As discovered in Chapter III, ammonium bicarbonate is a better buffer than calcium carbonate for anaerobic fermentations in the MixAlco process. Some concerns will be the role of pH in thermophilic fermentations. Both the chemical composition of the buffer and the pH in the buffer system are important factors for the fermentations. A previous conclusion in Chapter III showed that pH can play an important role in fermentation performance. If the pH is over 8.0, the anaerobic fermentation may fail. A question rises whether pH play a more important role than ammonium bicarbonate buffer itself. Maintaining a constant pH condition will help to answer this question.

The objective of this part is to continue comparing total product concentration in thermophilic fermentations using ammonium bicarbonate and calcium carbonate buffers. The experiments were designed to determine if pH or the presence of ammonium

bicarbonate is responsible for the high product concentrations. Paper was the best biomass subject for buffer comparison, because it was already chemically treated in paper pulping and therefore did not require additional chemical pretreatment to enhance digestibility. The pH in the fermentation broth was controlled around 7.0. This was designed to eliminate the potential pH effect and focus on the buffer comparison itself.

4.1.1 Materials and methods

As shown in Table 4-1, waste paper (16 g), chicken manure (16 g), nutrient mixture (0.3 g), anaerobic water (230 mL), and inocula (20 mL from previous ammonia bicarbonate buffered countercurrent fermentations) were added to initiate the fermentations. Iodoform solution (120 μL) with a concentration of 20 g/(L ethanol solution) was added to inhibit methane production. Calcium carbonate solid (Certified ACS grade, Fisher Scientific catalog #C64-500) and NH_4HCO_3 solid (Certified ACS grade, Fisher Scientific catalog #A643-500) were used as the pH buffer to adjust the desired pH in the fermentation broth. Urea (0.1 g, Certified ACS grade, Fisher Scientific catalog #U15-500) was initially added to calcium carbonate buffered fermentations, whereas no urea was used in ammonium bicarbonate buffered fermentations.

The pH control method used in this section is different from the pH control method used in Chapter III. In this chapter, the desired pH is 7.0. The effective pH buffer range of calcium carbonate does not cover 7.0, therefore additional lime ($Ca(OH)_2$) was used to help calcium carbonate to maintain the pH around 7.0. No lime was used in ammonia bicarbonate buffered fermentations. Ammonium bicarbonate solid (NH_4HCO_3) was the only pH buffer used for ammonium bicarbonate buffered fermentations. The fermentation broth pH was adjusted to around 7.0 (6.97–7.03), whenever the fermentor was opened. If the pH was more than, or very close to 7.0, no buffer (either $CaCO_3$/$Ca(OH)_2$ or NH_4HCO_3) was added in that case.

Table 4-1. Paper fermentation configures to compare effects of ammonium bicarbonate and calcium carbonate.

	Composition of biomass substrate	Buffer System	Inoculum
K1	16 g paper 4 g chicken manure	1 g initial $CaCO_3$ then fixed amount of 1 g/2 day $CaCO_3$ and variable $Ca(OH)_2$ to maintain pH around 7.0 (6.97–7.03)	20 mL inoculum from previous ammonia bicarbonate thermophilic countercurrent fermentations
K2	16 g paper 4 g chicken manure	1 g initial NH_4HCO_3 then variable NH_4HCO_3 to maintain pH around 7.0 (6.97–7.03)	20 mL inoculum from previous ammonia bicarbonate thermophilic countercurrent fermentations

4.1.2 Results and discussions

Total carboxylic acid concentration and pH for Fermentations K1 and K2 in Table 4-1 are shown in Figures 4-1 and 4-2. The pH in Figure 4-2 was measured prior to the pH adjustment with buffers, whenever the fermentors were opened. Figure 4-2 shows that the pH in both fermentations was well controlled around 7.0, which satisfies the required fixed pH conditions.

Figure 4-1 shows the product concentration increased with fermentation progress. There was similar performance for both fermentations in the initial 4 days. After the anaerobic microorganisms in the fermentation system grew, Fermentation K2 with ammonium bicarbonate started to exceed Fermentation K1 with calcium carbonate. The product concentration in Fermentation K1 reached 18.5 g/L in 25 days. In contrast, Fermentation K2 (with ammonium bicarbonate) harvested 26.5 g/L carboxylic acids in 25 days. There is a significant product concentration difference between two buffer systems. If pH is controlled around the desired 7.0, total product concentrations of fermentations using ammonium bicarbonate are still higher than those fermentation using calcium carbonate.

This experiment demonstrated that pH itself is not the only factor for high product concentration in ammonium bicarbonate fermentation. The cause is the difference of chemical properties between ammonium bicarbonate and calcium carbonate.

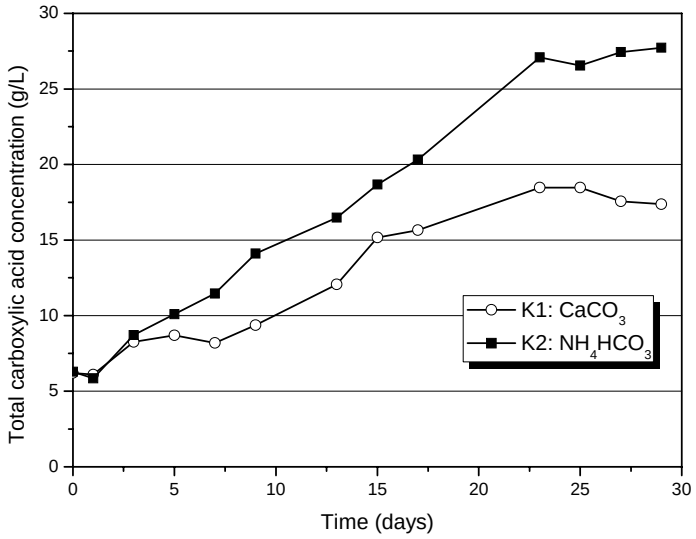


Figure 4-1. Total carboxylic acid concentration for paper fermentations under controlled pH. (○) Fermentation K1: $CaCO_3$ as main buffer, fixed $CaCO_3$ and varying $Ca(OH)_2$ to maintain pH around 7 (■) Fermentation K2: NH_4HCO_3 as main buffer, varying NH_4HCO_3 to maintain pH around 7.

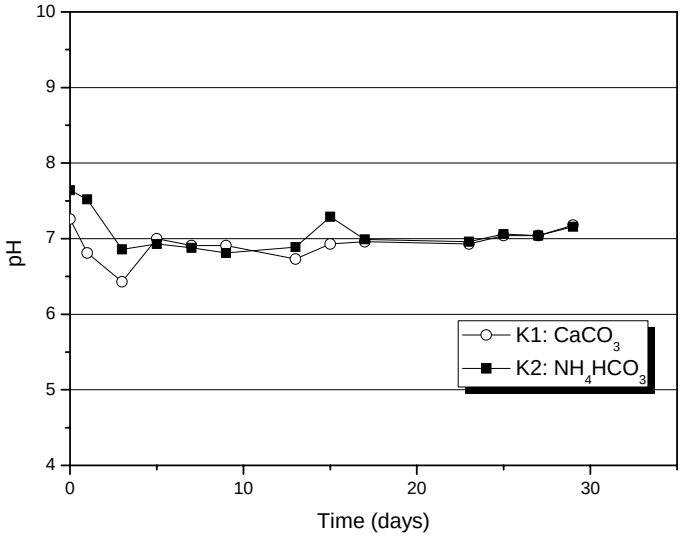


Figure 4-2. pH profiles for paper fermentations under controlled pH. (○) Fermentation K1: $CaCO_3$ as main buffer, fixed $CaCO_3$ and varying $Ca(OH)_2$ to maintain pH around 7 (■) Fermentation K2: NH_4HCO_3 as main buffer, varying NH_4HCO_3 to maintain pH around 7.

4.2 Ammonium bicarbonate as "methane inhibitor"

The role of ammonium bicarbonate in this improved anaerobic fermentation is not clear yet. Other than its role as a pH buffer, ammonium bicarbonate is also a nitrogen supplement to the microorganisms in fermentation system. This section describes some exploratory experiments. It is designed to determine whether ammonium bicarbonate serves as a "methane inhibitor" and to confirm if the traditional methane inhibitor (iodoform) is still required.

4.2.1 Materials and methods

Office paper and lime-treated bagasse were selected as the fermentation carbon sources in this section. Chicken manure was chosen as the nutrient source. The mixture of 80% biomass and 20% raw chicken manure was the initial substrates for all batch fermentations in this section (Table 4-2).

Fermentations K3 and K4 used paper as the substrate, whereas Fermentations K5, K6, and K7 used hot-lime-water-treated bagasse as fermentation substrate. Iodoform is the selected methane inhibitor, if required. Among the five different fermentation settings (each setting with a duplicate), Fermentations K3 and K5 were selected to contain methane inhibitor (iodoform), whereas Fermentations K4, K6, and K7 did not use iodoform during the whole fermentation. There was an additional 120 μL/4 day iodoform solution (20 g/L of iodoform dissolved in ethanol) added to Fermentations K3 and K5 to ensure sufficient methane inhibition. The total liquid volume in all fermentations was 250 mL. The pH in the fermentation broth was controlled around 7.0 (6.97–7.03). Inocula (20 mL) from previous ammonia bicarbonate thermophilic fermentations were used in all fermentations.

Table 4-2. Fermentation configures to examine "methane inhibitor" of ammonium bicarbonate.

	Methane inhibitor (iodoform)	biomass substrate	Buffer System	Total liquid volume (mL)	Inocula
K3	YES 120 µL	32 g paper, 8 g chicken manure	2 g initial NH_4HCO_3; then variable NH_4HCO_3 to maintain pH around 7.0 (6.97–7.03)	250	20 mL inocula from previous ammonia bicarbonate thermophilic fermentations
K4	NO	32 g paper, 8 g chicken manure	2 g initial NH_4HCO_3; then variable NH_4HCO_3 to maintain pH around 7.0 (6.97–7.03)	250	20 mL inocula from previous ammonia bicarbonate thermophilic fermentations
K5	YES 120 µL	32 g hot-lime-water-treated bagasse, 8 g chicken manure	2 g initial NH_4HCO_3; then variable NH_4HCO_3 to maintain pH around 7.0 (6.97–7.03)	250	20 mL inocula from previous ammonia bicarbonate thermophilic fermentations
K6	NO	32 g hot-lime-water-treated bagasse, 8 g chicken manure	2 g initial NH_4HCO_3; then variable NH_4HCO_3 to maintain pH around 7.0 (6.97–7.03)	250	20 mL inocula from previous ammonia bicarbonate thermophilic fermentations
K7	NO	48 g hot-lime-water-treated bagasse, 12 g chicken manure	3 g initial NH_4HCO_3; then variable NH_4HCO_3 to maintain pH around 7.0 (6.97–7.03)	250	20 mL inocula from previous ammonia bicarbonate thermophilic fermentations

4.2.2 Results and discussions

Total carboxylic acid concentrations and acetate contents for paper fermentations (K3 and K4) are shown in Figures 4-3 and 4-4. There was no methane detected in Fermentation K3, whereas there was around 3% methane detected in Fermentation K4 on Day 21. Methanogens in Fermentation K3 were completely inhibited by iodoform.

As shown in Figure 4-3, there was similar performance for both fermentations in the initial 10 days. Fermentation K3 with methane inhibitor achieved a little higher product concentration than Fermentation K4 without iodoform. The acid concentration in Fermentation K3 reached 41.6 g/L in 25 days. In contrast to the calcium carbonate buffered fermentation K1, Fermentation K4 (without methane inhibitor) produced 36.4 g/L carboxylic acids in 25 days. Although there was around 3% methane detected in Fermentation K4, the acid concentration in Fermentation K4 is acceptable and was not much different than Fermentation K3 using methane inhibitor.

The comparison of acetate contents in Figure 4-4 shows that there was no significant difference between Fermentations K3 and K4. Iodoform did not affect the acetate content in paper fermentations. In general, acetic acid is a direct substrate source for methanogens. If methanogens were not inhibited, acetic acid would be consumed and reduce the acetic acid concentration. The similar acetic acid concentration between Fermentations K3 and K4 suggests that ammonium bicarbonate is a weak "methane inhibitor." It did inhibit methanogens at some level in paper fermentations, but did not completely inhibit them.

Total acid concentrations of 45–52 g/L acid concentration were possible with ammonium bicarbonate buffered fermentations. The microorganisms were able to adapt to such high product concentrations. This is by far the highest product concentration achieved in batch fermentations, compared with the typical 26–30 g/L acid concentration in calcium carbonate buffered fermentations.

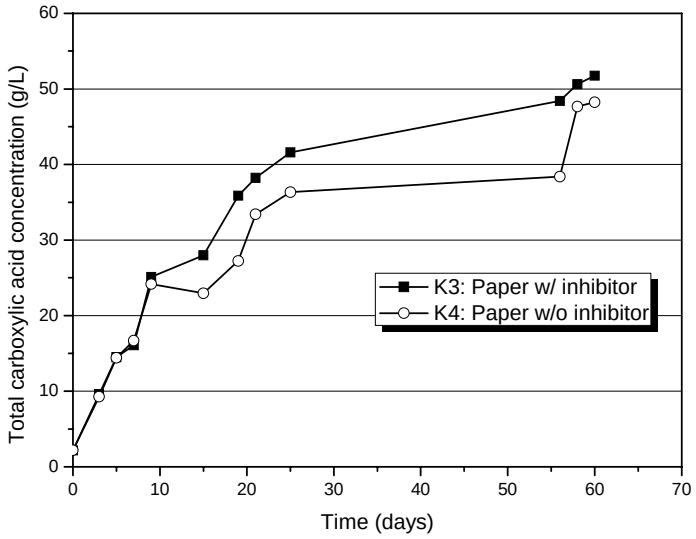


Figure 4-3. Total carboxylic acid concentration for paper fermentations under thermophilic conditions. Ammonium bicarbonate was used as buffer.

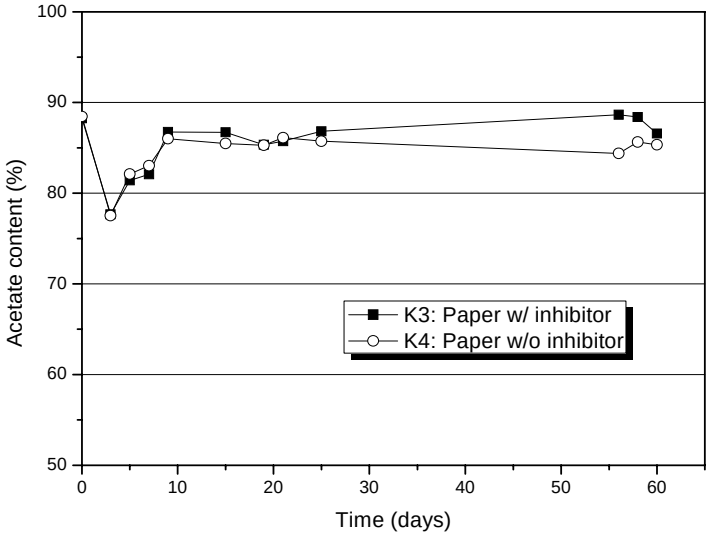


Figure 4-4. Acetate content in product from paper fermentations under thermophilic conditions. Ammonium bicarbonate was used as buffer.

Regarding methane inhibition, hot-lime-water-treated bagasse fermentation is different from paper fermentation. Ammonium bicarbonate in bagasse fermentations did a "weak" job in inhibiting methanogens. Although there was no methane detected before Day 10 in Fermentations K6 and K7 (without iodoform), there was around 5% methane detected on Day 16, and around 12% on Day 50. The methanogens in the hot-lime-water-treated bagasse fermentations were not inhibited by ammonium bicarbonate.

Total acid concentrations and acetate contents for bagasse fermentations are compared in Figures 4-5 and 4-6. The acetate contents were nearly the same in all three fermentations. Again, iodoform seems not to affect the acetic acid distribution in ammonium bicarbonate buffered fermentations. Figure 4-5 shows that Fermentation K5 with iodoform had the highest acid production. Both Fermentations K6 and K7 were impaired by methanogens. In 25 days, the acid concentration in Fermentation K5 reached 33.79 g/L, whereas Fermentation K6 (without methane inhibitor) reached 24.74 g/L. There was about 27% decrease of product concentration due to the lack of methane inhibitor. Furthermore, Fermentation K7 (initial 48 g bagasse w/o iodoform) achieved similar product concentration with Fermentation K5 (initial 32 g bagasse w/ iodoform). Thus, 50% more initial substrate only achieved similar product concentration. This also demonstrated that methanogens cannot be controlled to a reasonable level by ammonium bicarbonate only. The lack of methane inhibitor in bagasse fermentation resulted in a low product concentration even with the addition of ammonium bicarbonate.

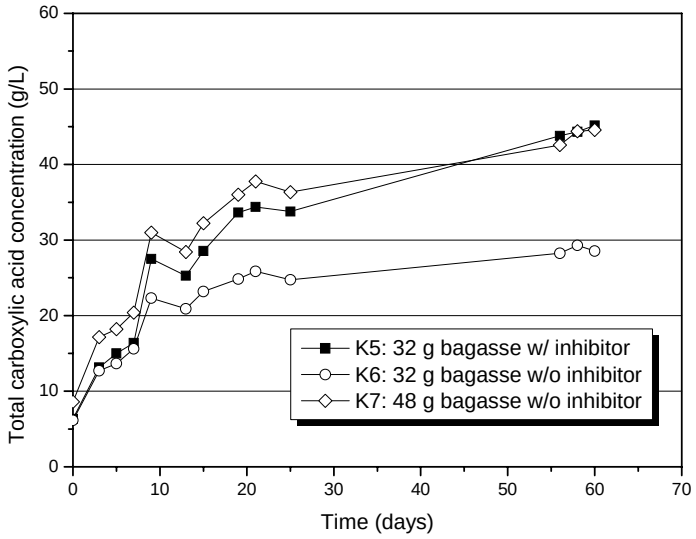


Figure 4-5. Total carboxylic acid concentration for bagasse fermentations under thermophilic conditions.

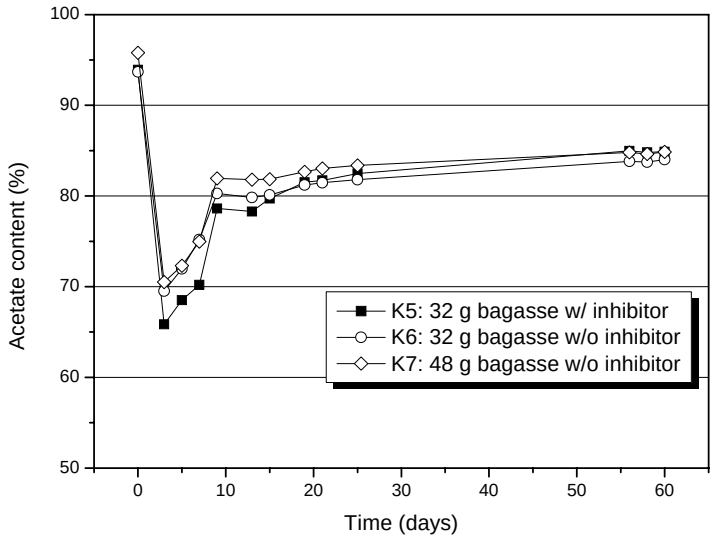


Figure 4-6. Acetate content in product from bagasse fermentations under thermophilic conditions. Ammonium bicarbonate was used as buffer.

Further personal communication with Andrea Forrest, a graduate student in our research group, shows that methane inhibitor is required for long-term bagasse fermentations with ammonium bicarbonate under thermophilic conditions. The initial operation of ammonium bicarbonate buffered fermentation with bagasse could not completely inhibit methanogens after 3 months operation and achieved a very low acid concentration at that time. Iodoform had to be added to the fermentation system to inhibit methanogens after that.

In conclusion, ammonium bicarbonate is not a strong "methane inhibitor." Methane inhibitor (iodoform) affects the acetic acid concentration, but not the acetate content, in all fermentation studied. Ammonium bicarbonate is at most a "weak" methane inhibitor and cannot completely inhibit methanogens. It is still unknown why ammonium bicarbonate had better methane inhibition performance in paper fermentations than bagasse fermentation.

4.3 Ammonia treatment for ammonium bicarbonate fermentation

Lime pretreatment is preferred in the traditional MixAlco process, because lime is inexpensive and safely handled. Lime is also recoverable in the MixAlco process. The so-called "lime loop" starts from fresh lime deployed in the lime treatment process. The introduced excess lime in the biomass treatment process will be neutralized and converted to calcium carbonate, which is the previously desired pH buffer for anaerobic fermentations. The resulting calcium carboxylate from the fermentation broth will be converted back to lime, which ends the "lime loop."

Lime treatment may not be suitable for the newly introduced ammonium bicarbonate buffer. Lime pretreatment of raw biomass introduces calcium salts to the anaerobic fermentations. The resulting fermentation product may not be pure ammonium carboxylate, but a mixture of ammonium and calcium carboxylate. This mixture may cause unexpected trouble when separating the desired product from fermentation effluents. For example, the resulting $CaCO_3$ could block membranes or foul heat exchangers.

Followed by the successful combination of lime pretreatment and calcium carbonate buffer, ammonia is a candidate alkali pretreatment agent for ammonium bicarbonate buffered fermentations. The logic is that the pair of lime ($Ca(OH)_2$) and calcium carbonate ($CaCO_3$) matches the pair of ammonia solution (NH_4OH) and ammonium bicarbonate (NH_4HCO_3). Aqueous ammonia solution is suitable for lignocellulosic biomass processing (Kim et al. 2003; Kim and Lee 2005a; Kim and Lee 2005b; Kim et al. 2006). Ammonia is a proven delignification reagent. It also performs other functions including hydrolysis of glucuronic acid ester crosslinks in biomass, cleaving of the lignin-hemicellulose bonds, and change of cellulose fiber structure.

In conclusion, if aqueous ammonia pretreatment can achieve similar biomass fermentation performance as lime pretreatment, we may expect efficient and low-cost product separation from anaerobic fermentations. The objective of this section is to start several preliminary experiments on ammonia pretreatment and validate if ammonia treatment is feasible.

4.3.1 Materials and methods

Paper is not used in this section, because paper does not require additional treatment before fermentation. Sugarcane bagasse is the desired biomass feedstock in this section.

Ammonia solution pretreatment

Long-term ammonia treatment and short-term ammonia treatment (Table 4-1) were used in this work. Table 4-3 compares the difference of "long-term" and "short-term" ammonia treatments.

Short-term treatment aims to harvest treated biomass in a reasonably short time (24 hours). Mild treatment temperature (55^{o}C) was maintained within a modified temperature-adjustable oven (Figure 4-7) in the short-term ammonia treatment. A self-constructed high-pressure reactor (Figure 4-8) is the desired reactor for short-term treatment.

Table 4-3. Comparison of "long-term" ammonia treatment and "short-term" ammonia pretreatment.

	Long-term pretreatment	Short-term pretreatment
Ammonia concentration	30%	10% or 30%
Pretreatment temperature	Room temperature	55^{o}C
Pretreatment container	1-L centrifuge bottle	Self-constructed high-pressure reactor
Temperature control	Roll-system, No temperature control required	Modified temperature-adjustable oven
Pretreatment time	12 days	1 day

Table 4-4. Ammonia solution treatment for sugarcane bagasse.

Sample	Treatment period	Alkaline agents used for pretreatment	Washing procedure	Post-pretreatment drying method
A	12 days	30% aquous ammonia NH_3	YES	105^{o}C oven for 2 days
B	1 day	30% aquous ammonia NH_3	YES	105^{o}C oven for 2 days
C	1 day	10% aquous ammonia NH_3	YES	105^{o}C oven for 2 days
D	0	NO	NO	105^{o}C oven for 2 days

A roller system (Figure 4-9) created mixing for the long-term treatment, whereas a room-temperature 1-L centrifuge bottle (Figure 4-10) was the desired reactor for long-term treatment. No temperature control was required in the long-term ammonia treatment.

Table 4-4 lists the ammonia-treated samples used to evaluate the performance of ammonium bicarbonate buffered fermentation in this section. Sample D is the control sample (no chemical treatment). Sample A is the long-term treated bagasse, whereas Samples B and C are the short-term treated bagasse. Different ammonia concentrations were used for Samples B and C. Compared with the low ammonia concentration (10%) for Sample C, high ammonia concentration (30%) was deployed with Sample B to check if the low ammonia usage is effective in the short-term ammonia treatment.

Figure 4-7. Modified temperature-adjustable oven for rapid batch ammonia pretreatment (24 hours).

Figure 4-8. Self-constructed high-pressure reactor for rapid batch ammonia pretreatment (24 hours).

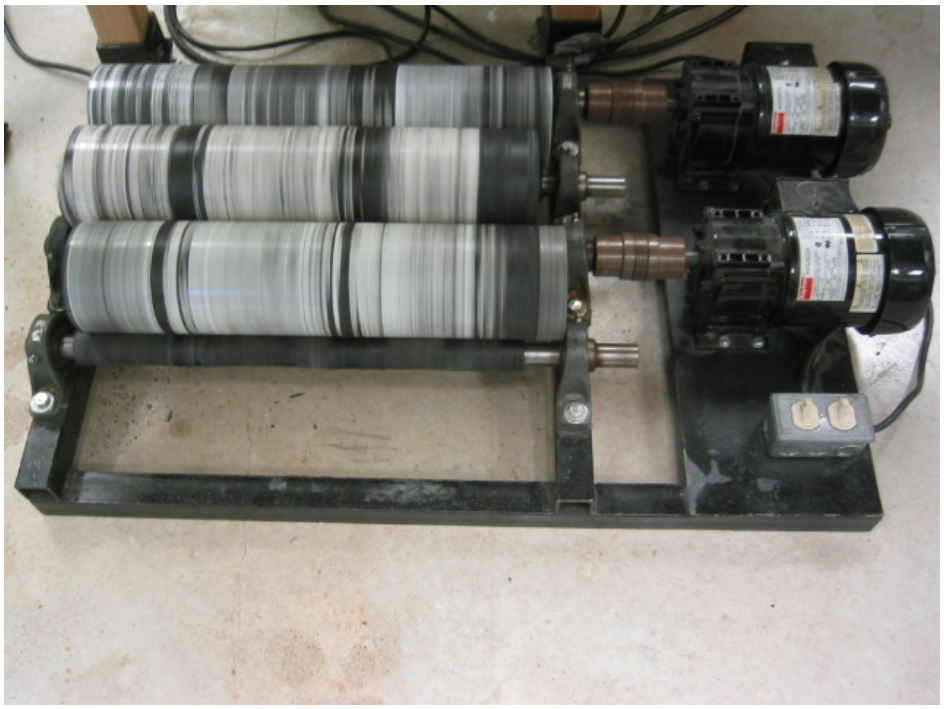


Figure 4-9. Roller system for long-term batch ammonia pretreatment (12 days).

Figure 4-10. Beckman 1-L centrifuge bottles (Nalgene brand NNI 3120-1010) for batch ammonia pretreatment.

Ammonium bicarbonate fermentation

Ammonia-treated bagasse was selected as the carbon sources of fermentations in this section (Table 4-5). Chicken manure was chosen as the nutrient source with the weight ratio of 80% bagasse/20% chicken manure.

Fermentation L4 was the control set using raw (untreated) bagasse. Fermentation L5 used the hot-lime-water-treated (100^{o}C and pretreatment time of 2 h) bagasse to compare the difference between lime treatment and ammonia treatment.

Fermentation L1 used long-term ammonia-treated bagasse, whereas Fermentations L2 and L3 used short-term ammonia-treated bagasse. Bagasse for Fermentations L1 and L2 was treated by a 30% ammonia solution. However, bagasse for Fermentation L3 was treated by a 10% ammonia solution. Iodoform solution (120 μL/2 days) was added to all fermentations to ensure methanogen inhibition. The pH in the fermentation broth was controlled around 7.0 (6.97–7.03) using ammonium bicarbonate. Inocula (20 mL) from previous ammonium bicarbonate buffered fermentation were used for all fermentations.

Table 4-5. Fermentation configures to examine ammonia treatment for further ammonium bicarbonate buffered fermentations.

	Treated bagasse	Chicken manure	Buffer	Total liquid volume (mL)	Inocula
L1*	16 g Sample A (30% long-term)	4 g	1 g initial NH_4HCO_3; then variable NH_4HCO_3 to maintain pH around 7.0 (6.97–7.03)	250	20 mL inocula from previous ammonia bicarbonate thermophilic fermentations
L2*	16 g Sample B (30% short-term)	4 g	1 g initial NH_4HCO_3; then variable NH_4HCO_3 to maintain pH around 7.0 (6.97–7.03)	250	20 mL inocula from previous ammonia bicarbonate thermophilic fermentations
L3*	16 g Sample C (10% short-term)	4 g	1 g initial NH_4HCO_3; then variable NH_4HCO_3 to maintain pH around 7.0 (6.97–7.03)	250	20 mL inocula from previous ammonia bicarbonate thermophilic fermentations
L4*	16 g Sample D (control set)	4 g	1 g initial NH_4HCO_3; then variable NH_4HCO_3 to maintain pH around 7.0 (6.97–7.03)	250	20 mL inocula from previous ammonia bicarbonate thermophilic fermentations
L5*	16 g lime-treated bagasse (100^{o}C and 2 h)	4 g	1 g initial NH_4HCO_3; then variable NH_4HCO_3 to maintain pH around 7.0 (6.97–7.03)	250	20 mL inocula from previous ammonia bicarbonate thermophilic fermentations

* Experiments were performed in duplicate and average results are reported.

Note: Sample A, B, C, and D refer to the same samples in Table 4-4.

4.3.2 Results and discussions

Total carboxylic acid concentrations and acetate contents for bagasse fermentations with different treatments (Fermentations L1, L2, and L4) are shown in Figures 4-11 and 4-12. Figure 4-11 shows that ammonia treatment is an effective treatment for sugarcane bagasse. Both long- and short-term treatments greatly enhanced the digestibility of biomass and obtained higher product concentrations, compared with the untreated bagasse (Sample D) in 24 days. Fermentation L1 (long-term ammonia treatment) produced 19.66 g/L in 24 days. Fermentation L2 (short-term ammonia treatment) obtained 18.09 g/L in 24 day. Both are higher than 10.02 g/L for untreated bagasse. Interestingly, the raw bagasse fermentation had higher acetate content (over 95%) compared to 85% for the ammonia-treated bagasse and 80-90% for lime-treated bagasse (Sections 4.1 and 4.3).

Figures 4-13 and 4-14 compare the total carboxylic acid concentrations and acetate contents for short-term treated bagasse with different initial ammonia concentrations. In short-term ammonia treatment at 55°C, 30% ammonia concentration is better than 10% ammonia concentration. As illustrated in Figure 4-13, the acid concentration in Fermentation L2 reached 18.09 g/L in 24 days. In contrast to Fermentation L2 (30% ammonia treated bagasse), Fermentation L3 (10% ammonia-treated bagasse) only produced 13.29 g/L carboxylic acids in 24 days. A higher acetate content (95%) was found in 10% ammonia-treated bagasse fermentation (Figure 4-13).

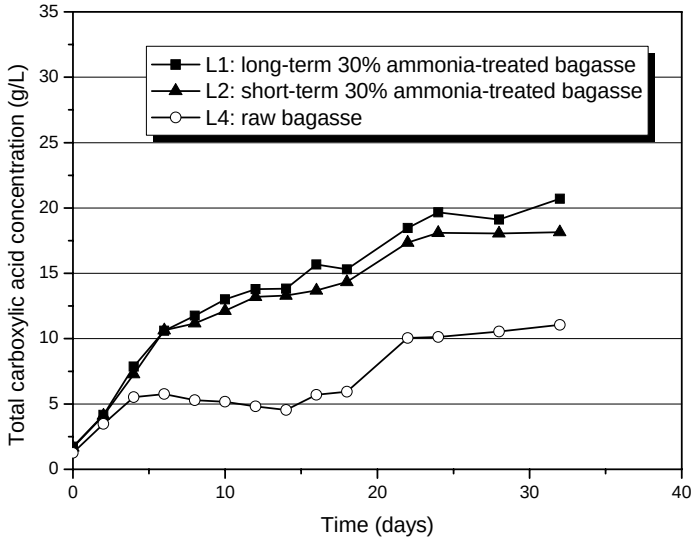


Figure 4-11. Total carboxylic acid concentration for ammonia-treated bagasse fermentations and untreated bagasse fermentations under thermophilic conditions.

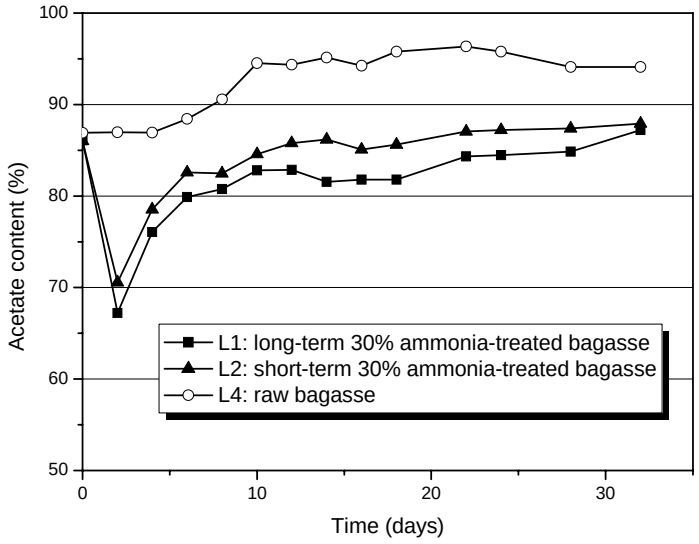


Figure 4-12. Acetate content in product for ammonia-treated bagasse fermentations and untreated bagasse fermentations under thermophilic conditions.

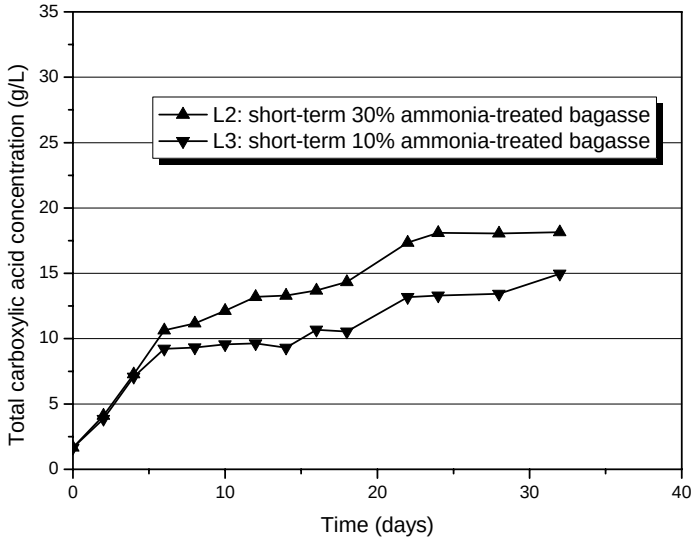


Figure 4-13. Total carboxylic acid concentration for 30% ammonia-treated bagasse fermentations and 10% ammonia-treated bagasse fermentations under thermophilic conditions.

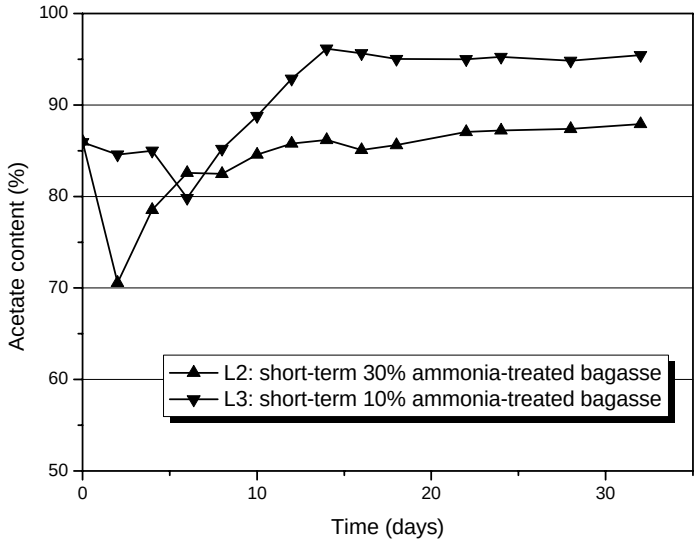


Figure 4-14. Acetate content in product for 30% ammonia-treated bagasse fermentations and 10% ammonia-treated bagasse fermentations under thermophilic conditions.

Total carboxylic acid concentrations and acetate contents for ammonia-treated bagasse with different pretreatment times are reported in Figures 4-15 and 4-16. Long-term 30% ammonia treatment at room temperature had similar performance as the short-term 30% ammonia treatment at 55°C. As illustrated in Figure 4-15, the acid concentration in Fermentation L2 reached 18.09 g/L in 24 days. In contrast to Fermentation L2 (short-term 30% ammonia-treated bagasse), Fermentation L1 (long-term 30% ammonia-treated bagasse) produced 19.66 g/L carboxylic acids in 24 days. This is a little better than the short-term ammonia treatment. Due to the similar acetate contents and product concentrations in ammonium bicarbonate buffered fermentation, 30% short-term ammonia treatment at 55°C will be selected as the only ammonia treatment method for future work, compared with the long-term ammonia treatment.

Figures 4-17 and 4-18 compare ammonia treatment with the hot-lime-water treatment. As illustrated in Figure 4-17, in 24 days Fermentation L2 (short-term 30% ammonia treated bagasse) reached 18.09 g/L, whereas Fermentation L5 (hot-lime-water-treated bagasse) produced 19.06 g/L carboxylic acids. There was no significant difference between the ammonia and lime treatments in this study. Both treatments led to similar product concentrations and acetate contents (around 85%) in ammonium bicarbonate buffered fermentations.

In summary, 30% short-term ammonia treatment at 55°C is a feasible biomass treatment for ammonium bicarbonate buffered fermentations and has a similar fermentation performance with the hot-lime-water treatment.

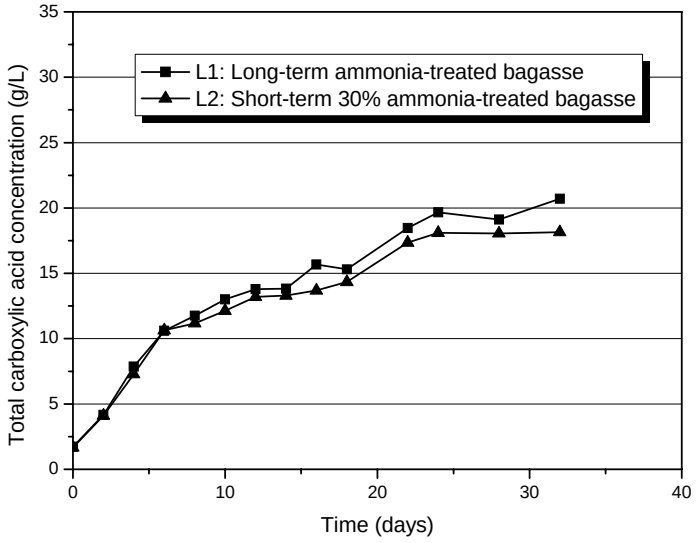


Figure 4-15. Total carboxylic acid concentration for long-term ammonia-treated bagasse fermentations and short-term 30% ammonia-treated bagasse fermentations under thermophilic conditions.

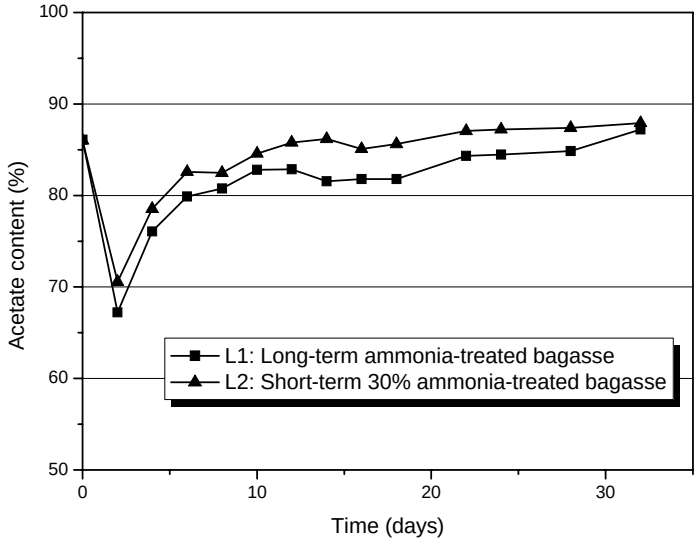


Figure 4-16. Acetate content in product for long-term ammonia-treated bagasse fermentations and short-term 30% ammonia-treated bagasse fermentations under thermophilic conditions.

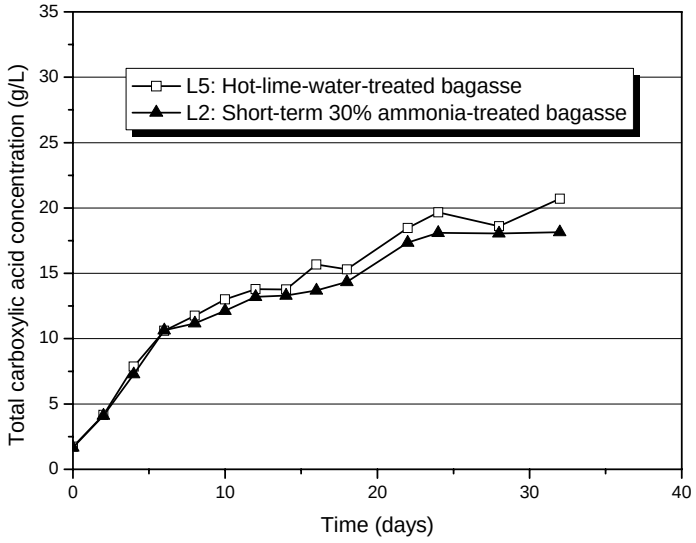


Figure 4-17. Total carboxylic acid concentration for hot-lime-water-treated bagasse fermentations and short-term 30% ammonia-treated bagasse fermentations under thermophilic conditions.

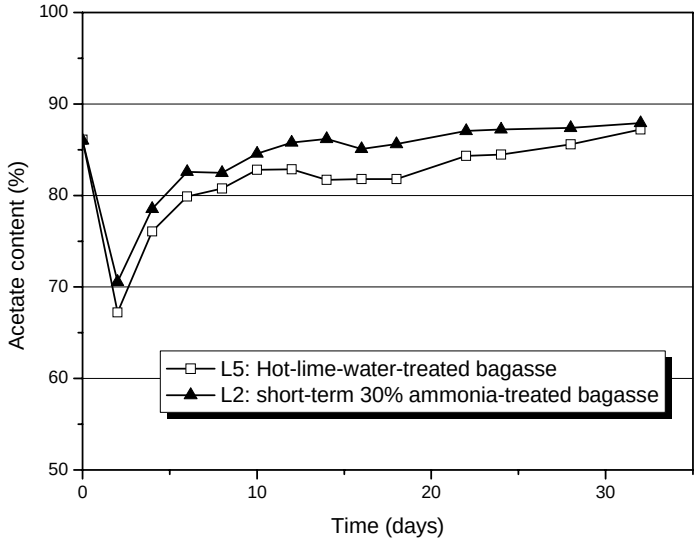


Figure 4-18. Acetate content in product for hot-lime-water-treated bagasse fermentations and short-term 30% ammonia-treated bagasse fermentations under thermophilic conditions.

4.4 Conclusions

This chapter continues the investigation of ammonium bicarbonate buffer. Some interesting conclusions follow:

1) Comparison of the ammonium bicarbonate and calcium carbonate under fixed pH conditions continue to show that ammonium bicarbonate is a better buffer.

2) Ammonium bicarbonate is a "weak" methane inhibitor. Around 3% methane was detected in the gas phase of the fermentation system showing that a strong methane inhibitor (e.g., iodoform) is still required in ammonium bicarbonate buffered fermentations.

3) Over 45 g/L acid concentration is possible with ammonium bicarbonate buffered fermentations. This is higher than the traditional 26–30 g/L acid concentration achieved in calcium carbonate buffered fermentations.

4) Ammonia solution treatment is a feasible biomass treatment for sugarcane bagasse. Anaerobic fermentations of the ammonia-treated bagasse have similar performance as fermentations of bagasse treated with hot-lime-water treatment, if ammonium bicarbonate is used as pH buffer.

5) Long-term (12 days) ammonia treatment at room temperature does not exceed the short-term (1 day) treatment in fermentation performance.

CHAPTER V

EFFECT OF RESIDUAL CALCIUM SALTS FROM LIME PRETREATMENT ON AMMONIUM BICARBONATE FERMENTATION

The objectives of this chapter follow:

a) To examine the effect of residual calcium salts in lime-treated bagasse on ammonium bicarbonate buffered fermentations. Three possible effects are assumed and will be validated.

b) To apply HCl solution to wash out the residual calcium salts from the lime-treated biomass.

c) To deploy three different biomass treatment methods: i) hot-lime-water treatment, ii) improved long-term lime treatment with air purging, and iii) ammonia solution treatment.

d) To validate whether a new biomass treatment (ammonia treatment) will be more effective than the hot-lime-water treatment. A better biomass treatment method may make the best use of ammonium bicarbonate buffer and possibly enhance the performance of the combined pretreatment and fermentation.

5.1 Introduction

As concluded in Chapter III, ammonium bicarbonate is a better buffer than calcium carbonate for anaerobic fermentations in the MixAlco process. Paper was initially used in the buffer comparison because it was already chemically treated in paper pulping and did not required pretreatment, whereas sugarcane bagasse must be pretreated. The experimental results in Chapter III are different for paper and sugarcane bagasse. For paper fermentations, the product concentration was nearly double, whereas it was only around 50–60% higher for bagasse fermentations. Although the compositional difference between paper and bagasse may result in this difference, residual calcium salts from lime pretreatment could be another important factor and therefore draws our interest. This chapter is dedicated to evaluating sources of residual calcium salts and their possible effects on ammonium bicarbonate buffered fermentation.

5.1.1 Composition of lime-treated biomass

In a typical MixAlco process, lime treatment of biomass is performed before anaerobic fermentation. Lime treatment can greatly enhance biomass digestibility and therefore improve fermentation performance. The preferred lime addition (0.1 g $Ca(OH)_2$/g raw biomass material) is in slight excess and ensures there is enough for biomass treatment. After the biomass is treated for the desired time, carbon dioxide is then bubbled into the biomass slurry to neutralize the excess lime until the pH is below 7.0. Therefore, the added lime will be converted to calcium salts mixed with the treated biomass. X-ray microanalysis of untreated bagasse (Figure 5-1) and lime-treated bagasse (Figure 5-2) shows that large amounts of calcium salts still remain in treated bagasse (Lopez et al. 2000).

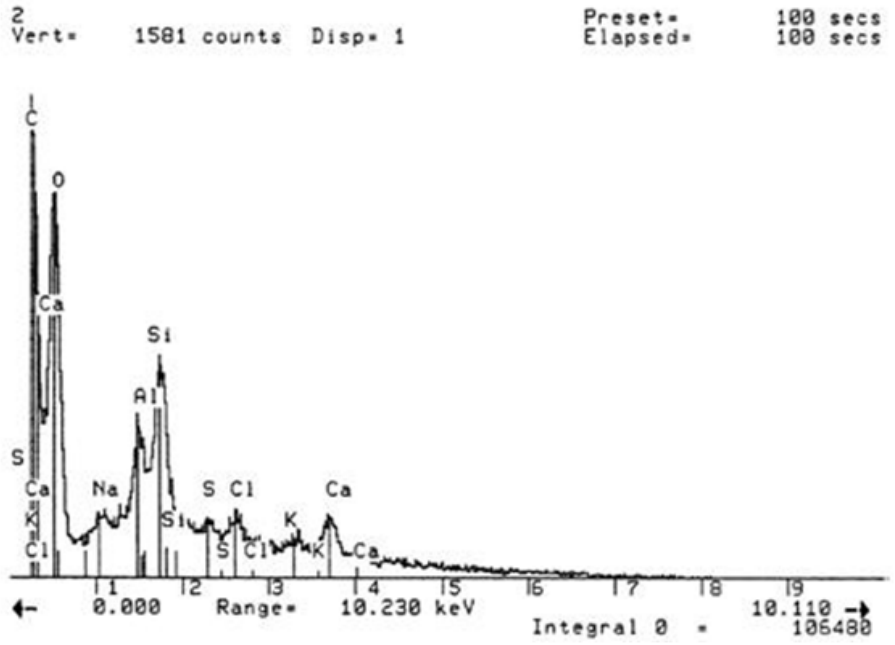


Figure 5-1. X-ray microanalysis results on untreated sugarcane bagasse (Lopez et al. 2000).

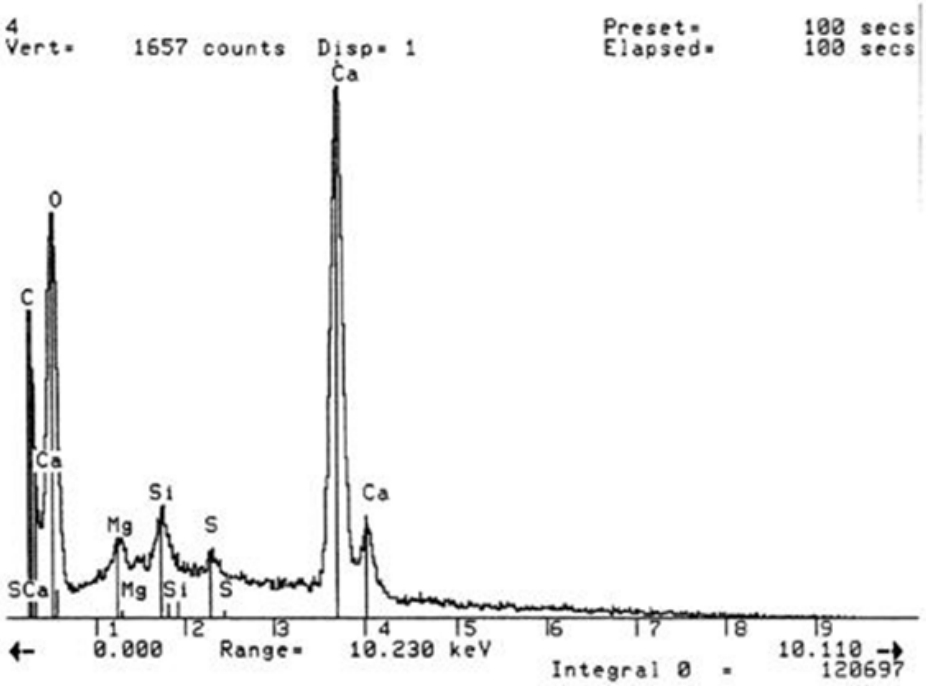


Figure 5-2. X-ray microanalysis results on lime-treated sugarcane bagasse (Lopez et al. 2000).

To calculate the weight ratio of residual calcium salts in the lime-treated biomass, it was assumed that the residual calcium salts come from lime addition (0.1 g $Ca(OH)_2$/g biomass). The weight ratio was calculated by the mass balance of calcium in the hot-lime-water treatment.

In theory, in lime treatment, 100% of calcium salt from lime ($Ca(OH)_2$) will stay in the solid phase of the harvested treated biomass, because the treatment process is a closed system and no calcium salts escape from lime treatment process. Although there may be calcium acetate existing in the treated biomass, the estimated weight ratio of calcium salts residing in the treated biomass can be calculated based on calcium carbonate (Equations 5-1 and 5-2), if all calcium salts are assumed to be in the form of calcium carbonate.

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O \tag{5-1}$$

Weight ratio of residual calcium salts in lime-treated biomass

$$= \frac{0.1\ \text{g lime} \times \dfrac{\text{Molecular weight of } CaCO_3}{\text{Molecular weight of } Ca(OH)_2}}{1\ \text{g dry raw biomass} + 0.1\ \text{g lime} \times \dfrac{\text{Molecular weight of } CaCO_3}{\text{Molecular weight of } Ca(OH)_2}}$$

$$= \frac{10\% \times \dfrac{100}{74}}{1 + 10\% \times \dfrac{100}{74}} = 11.9\% \tag{5-2}$$

Therefore, the lime-treated biomass is a mixture of biomass and calcium salts with an estimated weight ratio of 11.9% residual calcium salts (based on $CaCO_3$).

5.1.2 Possible effects of residual calcium salts

After pretreatment, the harvested biomass is a mixture of treated biomass and residual calcium salts (solid phase). When the treated biomass is fed to the anaerobic fermentor, the residual calcium salts may affect the performance of anaerobic fermentations buffered by ammonium bicarbonate in three different ways: a) mixed effects of calcium carbonate and ammonium bicarbonate may weaken the benefit of ammonium bicarbonate, b) residual calcium salts in the solid phase may block anaerobic microorganisms entering micropores of the treated biomass and therefore hinder fermentation performance, and c) possible excessive soluble calcium salts in fermentation broths may impair the ability of microorganisms to maintain ion gradients across biological membranes and thus inhibit biomass digestion by anaerobic microorganisms.

Mixed buffer effect of calcium carbonate and ammonium bicarbonate

As concluded in Chapters III and IV, extensive comparisons of calcium carbonate and ammonium bicarbonate buffers show that ammonium bicarbonate is better. The total carboxylic acid concentration from ammonium bicarbonate buffered fermentations of lime-treated bagasse can be nearly 50–60% above calcium carbonate buffered fermentations. The 9.1% weight ratio of ammonium bicarbonate (2 g buffer/20 g biomass) is sufficient to significantly increase product concentration in the fermentation broth in 16 days (Chapter III). Therefore, the estimated weight ratio of calcium salts presented in lime-treated biomass (11.9%) is nearly the same as the ammonium bicarbonate used in the fermentations (9.1%). This mixture of ammonium bicarbonate and calcium carbonate may offset the benefit of ammonia bicarbonate, because calcium carbonate serves as a pH buffer and may therefore reduce usage of ammonia bicarbonate. The concern is that the presence of calcium in a mixture of ammonium bicarbonate and calcium carbonate may offset the beneficial effect of ammonium bicarbonate alone.

Biomass blocked by residual calcium salts

Microstructure comparison of untreated and lime-treated sugarcane bagasse shows that the surface of lime-treated bagasse is covered by calcium carbonate particles and microparticles. Lopez et al. (2000) compared the SEM (Scanning Electron Microscopy) 500X images of raw bagasse (Figure 5-3) with lime-treated bagasse (Figure 5-4), and determined that lime treatment modifies the sugarcane bagasse surface by depositing calcium carbonate all over the fibers. Cesar Granda (2004) took more than 4 hours to wash out around 0.3 g of calcium from 3.0 g lime-treated bagasse during his measurements of lime consumptions during treatment. He concluded that calcium salts produced during lime treatment are difficult to wash out. It is possible that the produced calcium salts stick to the biomass surface and block biomass micropores. This "blockage" may decrease the accessibility of biomass to anaerobic microorganisms during fermentations and therefore impair fermentation performance. In a word, the residual calcium salts in lime-treated biomass may impede ammonium bicarbonate buffered fermentations.

Toxicity of excessive calcium salts residual in fermentation broth

Another issue is the soluble calcium salts remaining in the fermentation broth. Anaerobic fermentation in the MixAlco process is an acid-producing process. The produced acids can react with residual calcium salts and convert insoluble calcium salts to soluble calcium salts. Although soluble calcium salts may not affect calcium carbonate buffered fermentations, they could inhibit the anaerobic microorganisms growing in ammonium bicarbonate buffer. Possibly, excessive soluble calcium salts in the fermentation broths may impair the ability of microorganisms to maintain ion gradients across biological membranes, and thus inhibit their ability to digest the substrate.

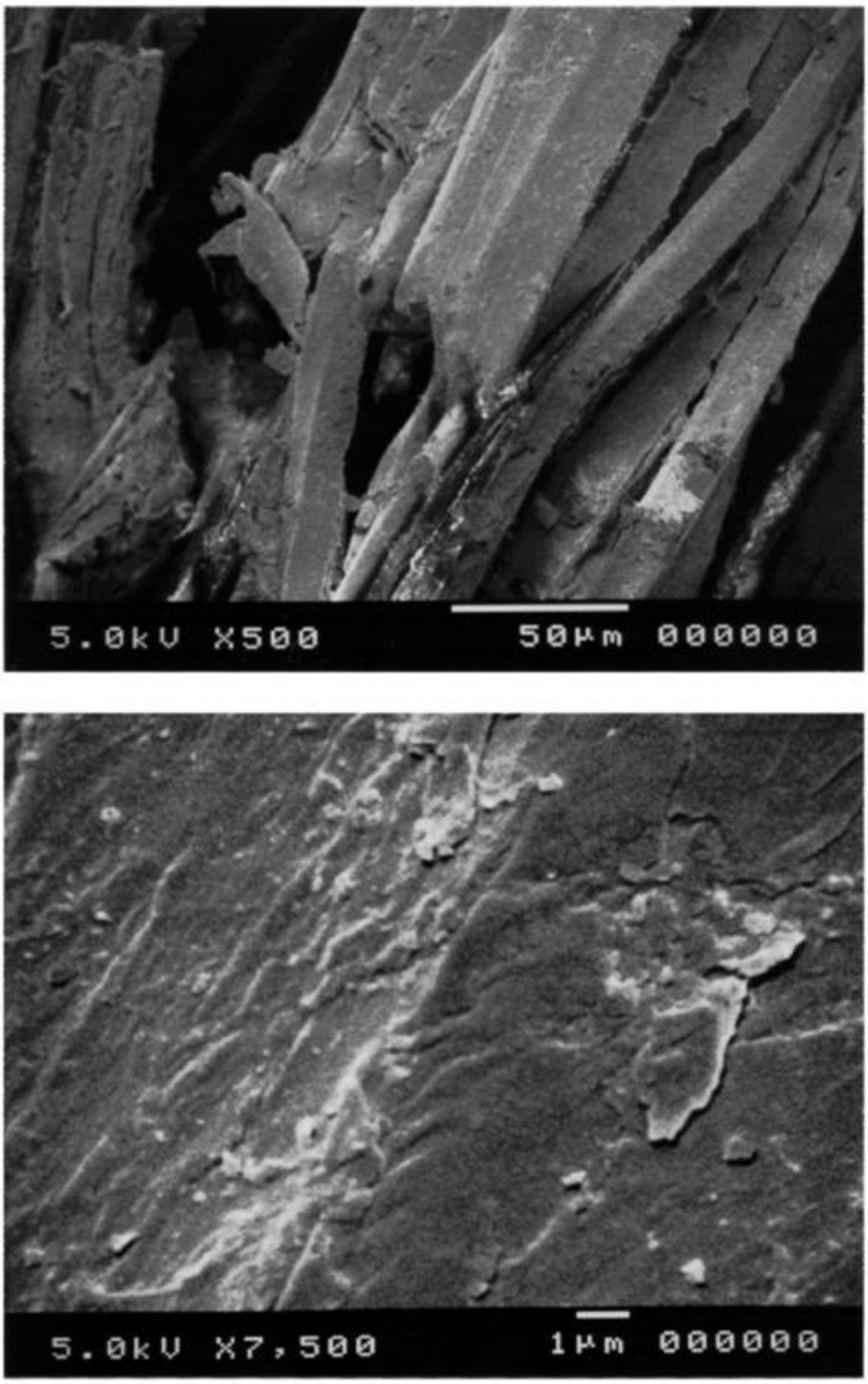


Figure 5-3. SEM images of untreated sugarcane bagasse (Lopez et al. 2000).

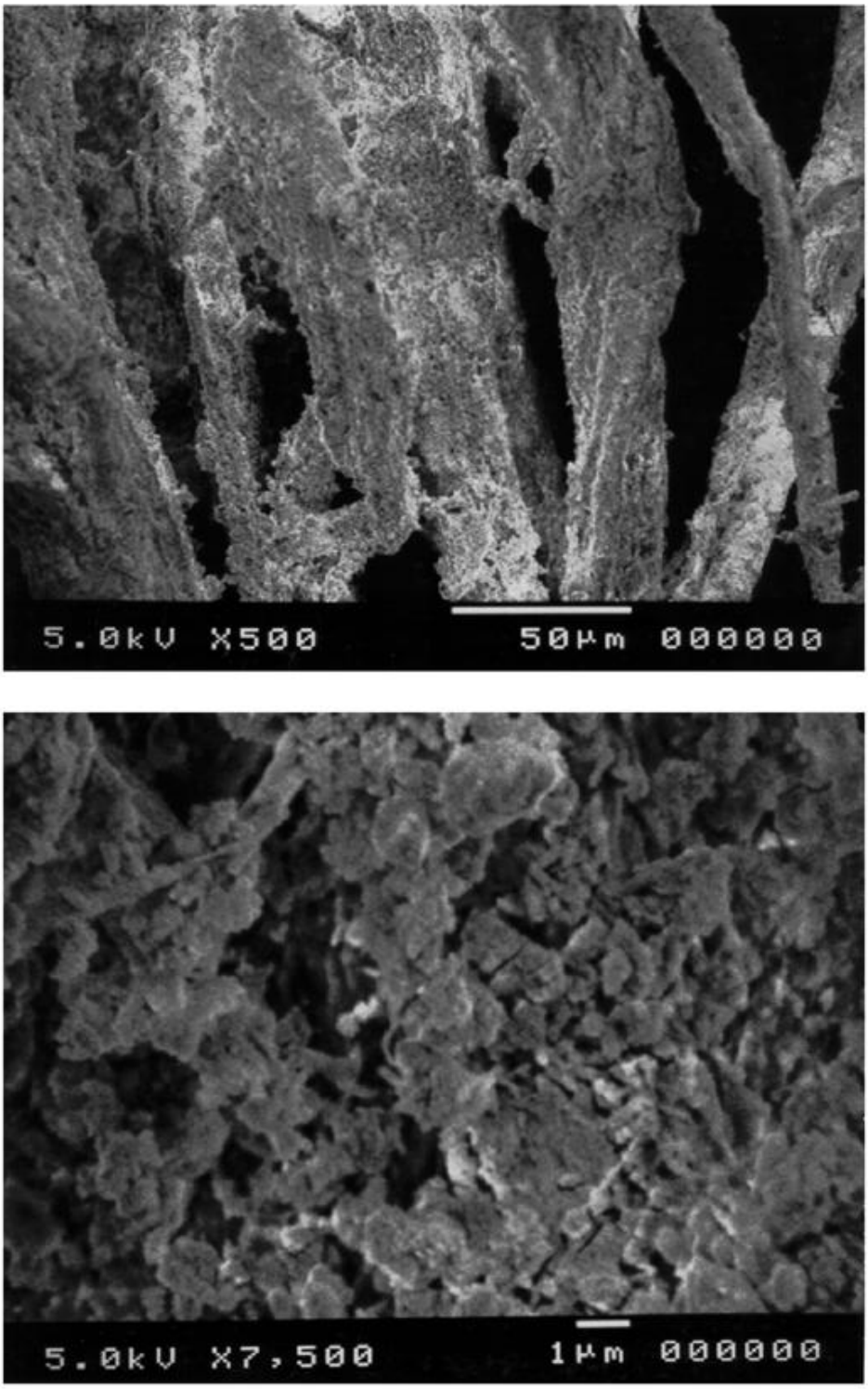


Figure 5-4. SEM images of lime-treated sugarcane bagasse (Lopez et al. 2000).

The possible toxic effect of residual calcium salts to the microorganisms is not directly investigated in this chapter, because this chapter is mainly concerned with the engineering application of anaerobic fermentations. The biologic feature of the microorganisms (e.g., cell density change) will not be investigated in this study.

The residual calcium salt in the treated biomass is a potential issue if ammonium bicarbonate is selected as the main pH buffer for anaerobic fermentations. This chapter is therefore designed to check possible effects of residual calcium salts in the anaerobic fermentations of lime-treated biomass. The results in this chapter are expected to provide some fundamental information on improving pretreatment conditions (e.g., using ammonia pretreatment as an alternative pretreatment method other than hot-lime-water treatment) to make the best use of the new ammonium bicarbonate buffer for anaerobic fermentations.

In this chapter, several modified lime-treatment methods are described with focus on different neutralization agents and procedures for washing out residual calcium salts. Different fermentation configurations will be performed to compare thermophilic fermentation performance and evaluate effects of residual calcium salts in the treated bagasse. In addition, three different biomass treatments (i.e., hot-lime-water treatment, air-lime treatment, and ammonia treatment) will be used to further evaluate the effect of residual calcium salts on fermentation performance.

5.2 Materials and methods

Table 5-1 summarizes the pretreatment and fermentation conditions used in this chapter, whereas Table 5-2 lists several different traditional or modified lime treatment methods: Sample A is raw (i.e., untreated) bagasse; Sample B is hot-lime-treated bagasse with carbon dioxide neutralization; Samples C, D, and E are hot-lime-water-treated bagasse with modifications of the neutralization agent (HCl in this case); Samples F and G are ammonia-treated bagasse; and Sample H is air-lime-treated bagasse.

5.2.1 Biomass pretreatment

Sample B: Hot-lime-water pretreatment procedure (carbon dioxide neutralizing without washing)

Sample B was pretreated using hot lime water, a widely used procedure (Agbogbo 2005; Aiello Mazzarri 2002; Thanakoses 2002). Raw sugarcane bagasse, deionized water and lime (0.1 g $Ca(OH)_2$/g dry biomass) were fully mixed and heated to boiling at 100^{o}C. After cooking for 2 hours, the biomass slurry was cooled to room temperature. Then, CO_2 gas was bubbled into the biomass slurry to neutralize excess lime. The slurry was dried in the oven at 105^{o}C for 2 days.

Samples C, D, and E: Modified lime pretreatment procedure (HCl neutralizing with water washing)

A modified lime-treatment procedure was deployed with Samples C, D, and E. Carbon dioxide gas, hydrogen chloride solution (hydrochloric acid, HCl), and acetic acid solution (CH_3COOH) are conventional neutralization agents used in our research group for lime pretreatment. Acetic acid (CH_3COOH) is not used in this chapter, because acetic acid washing procedure may introduce unwanted CH_3COO^- to the fermentation process. Any acetic acid remaining from the neutralization would artificially increase acetic acid in fermentation broth, thus making comparisons complex. Therefore, an HCl solution was used to replace the widely used CO_2 gas as a neutralizing agent in this modification of lime treatment.

Table 5-1. Matrix table for investigations on residual calcium salts.

Operating conditions		Case	Used
Substrate (nutrient source)		Chicken manure	√
Substrate (carbon source)		Bagasse	√
		Paper	√
Pretreatment (Bagasse)	Chemical	Lime solid, $Ca(OH)_2$	√
		Aqueous ammonia, $NH_3 + H_2O$	√
	Temperature	55°C	√
		100°C	√
		Room temperature (20–25°C)	
	Time	2 hours	√
		1 day	
		12 days	
		2 month	√
	Neutralization	Carbon dioxide, CO_2	√
		Hydrogen chloride, HCl	√
		Acetic acid, CH_3COOH	
		D.I. water washing, no chemicals	√
	Drying method	105°C Oven (2 d)	√
		Room temperature hood (2 d)	
Fermentation	Temperature	Thermophilic conditions (55°C)	√
		Mesophilic conditions (40°C)	
	pH buffer	Ammonium bicarbonate, NH_4HCO_3	√
		Calcium carbonate, $CaCO_3$	√
	Methane inhibitor	Iodoform	√
	Inoculum source	Original (unadapted) marine inoculum	
		Adapted marine inoculum from previous countercurrent fermentation	√
		Original (unadapted) lake inoculum	

Table 5-2. Different pretreatment procedures used for sugarcane bagasse.

Bagasse Sample	Alkaline agents used in treatment process	Neutralization Agents	Calcium salts washing procedure	Post-treatment drying method	Used for fermentations in this chapter
A	NO	NO	NO	105°C oven for 2 days	NO
B	H_2O + lime, $Ca(OH)_2$	CO_2 gas	NO	105°C oven for 2 days	YES
C	H_2O + lime, $Ca(OH)_2$	5-M HCl	NO	105°C oven for 2 days	NO
D	H_2O + lime, $Ca(OH)_2$	5-M HCl	YES	Air-dry in hood at room temperature	NO
E	H_2O + lime, $Ca(OH)_2$	5-M HCl	YES	105°C oven for 2 days	YES
F	Ammonia solution $NH_3 + H_2O$	NO	YES	Air-dry in hood at room temperature	NO
G	Ammonia solution $NH_3 + H_2O$	NO	NO	105°C oven for 2 days	YES
H	Air-lime $Ca(OH)_2$; long-term treatment with air purging	Acetic acid	YES	105°C oven for 2 days	YES

Raw sugarcane bagasse, water, and a desired amount of lime (0.1 g $Ca(OH)_2$/dry biomass) were fully mixed and heated to boiling at 100^{o}C. After cooking 2 hours, the biomass slurry was cooled to room temperature. Hydrochlolic acid solution was slowly and step-by-step added until neutral pH (7.0) was achieved. The neutralized biomass was dried or further washed to remove calcium salts. Two washing techniques have been used in our research group: (1) Filter-rinsing cycle and (2) Mix-stir-centrifuge-mix cycle. Sample E was prepared using the second procedure.

(1) Filter-rinsing cycle

After 2 h of stirring, the bagasse was separated by filtration and rinsed with distilled water until neutral pH was achieved (five washes, on average). After rinsing, the bagasse was dried in an oven for two days at 105^{o}C. This procedure was not used in this chapter.

(2) Mix-stir-centrifuge-mix cycle

A mix-stir-centrifuge-mix cycle starts when the pretreated biomass and desired amount of distilled water were added to a 1-L centrifuge bottle. After 4.0 h of stirring with a stir bar using a Corning stirrer, the pH was measured. The bagasse slurry sealed in the centrifuge bottle was centrifuged in a Beckman floor centrifuge machine (Model# J-6B) at a rotating speed of 4000 rpm for 25 minutes. After the solid and liquid were separated, the liquid was discarded and the desired amount of distilled water was added again to the centrifuge bottle. This ended a mix-stir-centrifuge-mix cycle. The mix-stir-centrifuge-mix cycles were terminated if the pH or color remained unchanged (six washes, on average). After the mix-stir-centrifuge-mix cycles, the separated wet cake was removed from the centrifuge bottle and dried for at least 2 days. This procedure was used in this chapter.

Samples F and G: Ammonia pretreatment (no neutralizing but with water washing)

Short-term 30% ammonia treatment at 55^{o}C was used to prepare Samples F and G.

Sample H: Air-lime treatment procedure (lime treatment with air purge)

An improved lime treatment was utilized for Sample H. Raw sugar cane baggase, water and desired amount of lime (0.3 g $Ca(OH)_2$/g dry biomass) were fully mixed and packed in the self-constructed long-term lime treatment bed. Air was continuously flushed into the pretreatment system. After 2 months, the biomass slurry was cooled to room temperature. Once the biomass was cooled, acetic acid was titrated into the biomass slurry to neutralize the excess lime. The slurry was dried in the oven at 105^{o}C for 2 days. Dried treated bagasse (Sample H) was used for further fermentation. Different from the long-term air-lime treated bagasse used in Chapter IX, Sample H was taken from Jones's long-term lime treatment batch (Jones 2007)

5.2.2 Fermentations

Paper (16 g) or treated bagasse (16 g), chicken manure (4 g, from Poultry Science Center, Texas A&M University, College Station, TX, 77843), nutrient mixture (0.3 g), anaerobic water (230 mL), and inocula (20 mL from previous ammonia bicarbonate countercurrent fermentations) were added to initiate the fermentations. Iodoform solution (120 μL of 20 g/L iodoform in ethanol solution) was added to inhibit methane production. $CaCO_3$ solid (Certified ACS grade, Fisher Scientific catalog #C64-500) and NH_4HCO_3 solid (Certified grade, Fisher Scientific catalog #A643-500) were used as buffer to adjust pH. An Orion portable full-featured pH/temperature meter (Model #230A) including the Triode^{TM} 3-in-1 combination pH/ATC electrode (Model #58819-91) with BNC connector was used for a rapid pH measurement of the fermentations.

5.3 Results and discussions

5.3.1 Residual calcium salts in different treatments

The residual calcium salts were identified by two ways: a) the mass concentration of calcium composition in various treated biomass, and b) the residual soluble carboxylate salt concentration.

Residual calcium salts in lime-treated biomass

Table 5-3 lists the metal composition of the raw bagasse and the pretreated bagasse with different neutralization methods. The metal composition of the wash liquid is also included in Table 5-3. Calcium composition is the major concern in this chapter. All solid and liquid samples were tested by Soil, Water, and Forage Testing Laboratory (http://soiltesting.tamu.edu/) in Texas A&M University (345 Heep Center, TAMU, College Station, TX 77843, contact phone 979-845-4816).

The calcium composition in Table 5-3 confirms that there is large amount of calcium (46,157 ppm) in the lime-treated bagasse (Sample B), because there is not much calcium (1,658 ppm) in the raw bagasse (Sample A).

A 24-hour HCl washing was determined to completely remove calcium for lime-treated bagasse. The color of the 5^{th} and 6^{th} washing liquid was clear, whereas the 1^{st} washing liquid was yellowish. The pH was stable after 5^{th} HCl wash procedure. The pH in the 5^{th} wash liquid was nearly identical to the pH in the 6^{th} wash procedure. Furthermore, the calcium content in the 5^{th} wash liquid (42.06 ppm in Sample M) as illustrated in Table 5-3 is very close to the calcium content in the 6^{th} wash-out liquid (26.47 ppm in Sample N). Because every wash process takes 4 hours, the 6^{th} HCl wash loop (i.e., 24 hours washing) can be assumed as a complete calcium salt washing. No additional HCl wash was performed after the 6^{th} wash in this study.

Table 5-3. Metal composition difference of lime-treated bagasse solid and HCl wash liquid.

	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)	Zn (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)
Raw bagasse (**Sample A**)	124.2	380	1658	238	1971	19.3	515	2.06	13.7
Lime-pretreated bagasse (**Sample B**)[1]	118.6	469	46157	355	2501	20.9	484.3	2.56	14.1
Lime-pretreated bagasse (**Sample C**)[2]	122.1	537	52452	427	2925	24	450.4	3.76	14.3
Lime-pretreated bagasse (**Sample E)**[3]	33.99	103	5846	123	1074	24.1	456.4	2.05	9.64
Wash liquid sample (**Sample M**, 5th HCl Wash)	0.782	6.39	42.06	2.05	67	0.1	1.65	0.06	0.17
Wash liquid sample (**Sample N**, 6th HCl Wash)	0.292	6.43	26.47	2.3	74.1	0.1	1.432	0.06	0.18

Note: Details of Samples A, B, C, and E refer to samples in Table 5-2.

[1] Sample B refers to hot-lime-water pretreatment using CO_2 to neutralize without additional washing procedure
[2] Sample C refers to hot-lime-water pretreatment using HCl to neutralize without additional washing procedure
[3] Sample E refers to hot-lime-water pretreatment using HCl to neutralize with additional washing procedure (6 washes)

The HCl washing procedure could not fully remove the newly introduced calcium from lime treatment. The calcium composition in the hot-lime-water-treated bagasse was 46,157 ppm as illustrated in Table 5-3, whereas the calcium composition in the 6th HCl washed lime-treated bagasse was 5,846 ppm. There is still around 13% of calcium that could not be removed by washing and remained in the treated bagasse (solid phase). There is likely some bound calcium in the micropores of the treated bagasse. Similar results were also reported using SEM imagine technique (Lopez et al. 2000).

Residual carboxylate salts in lime-treated biomass

Residual calcium salts were also measured as carboxylic acids. The lime-treated bagasse/water mixture with the same weight ratio (i.e., 4 g/62.5 mL) used in fermentations was fully mixed using the stirrer for 2 hours. Clear centrifuged liquid (3 mL) was taken from the mixture of treated bagasse and water. This liquid sample was prepared and the total acid concentrations were measured by gas chromatography as described in Chapter II.

Figures 5-5 and 5-6 show the detected residual soluble carboxylic acids in the lime-treated bagasse using different neutralization methods. Acetic acid was the only carboxylic acid detected in hot-lime-water-treated bagasse as shown in Figure 5-7. No other C3–C7 carboxylic acids were detected. Four sets of liquid samples were analyzed for the residual calcium carboxylate concentration and the results are reported in Table 5-4. Samples 1–4 in Table 5-4 were an average of 2.05 g acids /L liquid (or 0.032 g acids/g dry treated bagasse). This is around 24% of the total estimated residual calcium salts (0.135 g calcium carbonate/g dry treated bagasse). Therefore, the residual calcium salts are a mixture of calcium acetate and calcium carbonate. Furthermore, 2.05 g acids/L fermentation broth from the hot-lime-water-treated bagasse could be a significant source when fermentations utilize the bagasse.

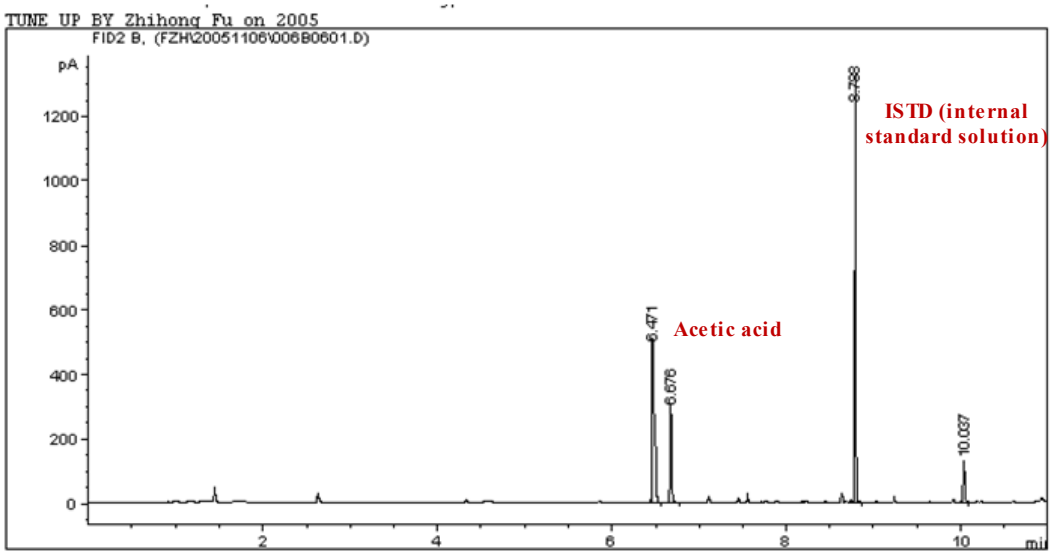


Figure 5-5. GC output for hot-lime-water-treated bagasse with carbon dioxide neutralization.

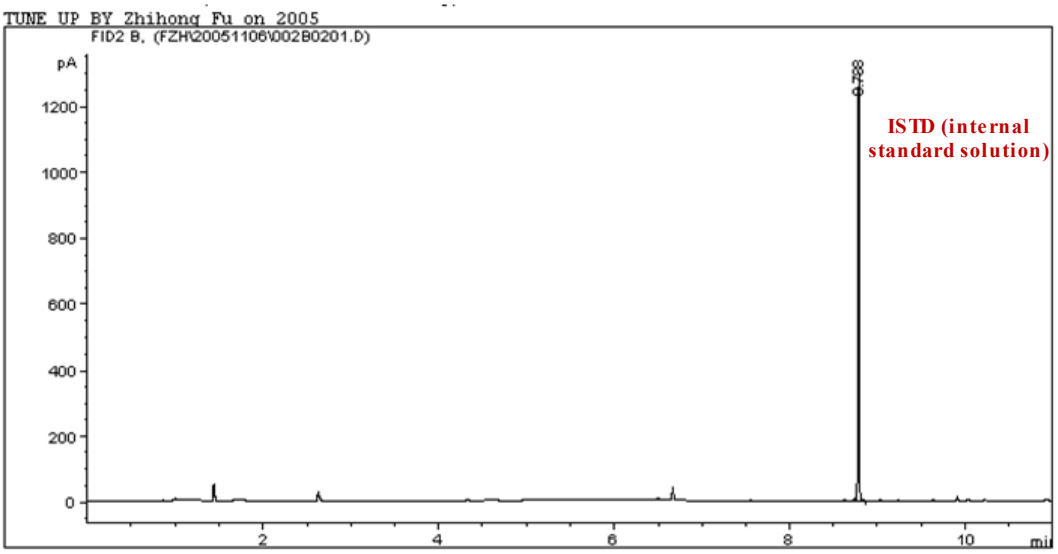


Figure 5-6. GC output for hot-lime-water-treated bagasse with HCl neutralization and D.I. water washing procedure.

Table 5-4. Detected residual carboxylic acids in liquid samples from treated bagasse.

Bagasse samples		Detected acetic acid concentration (g/L)	Detected total carboxylic acid concentration (g/L)
CO_2-no-wash procedures	S1	2.04	2.04
	S2	2.05	2.05
	S3	2.07	2.07
	S4	2.05	2.05
HCl washing procedures	S5	0	0
	S6	0	0
	S7	0	0
	S8	0	0

Note: All of detected carboxylic acid concentration is for the treated bagasse/water mixture with same weight ratio as that in fermentations.

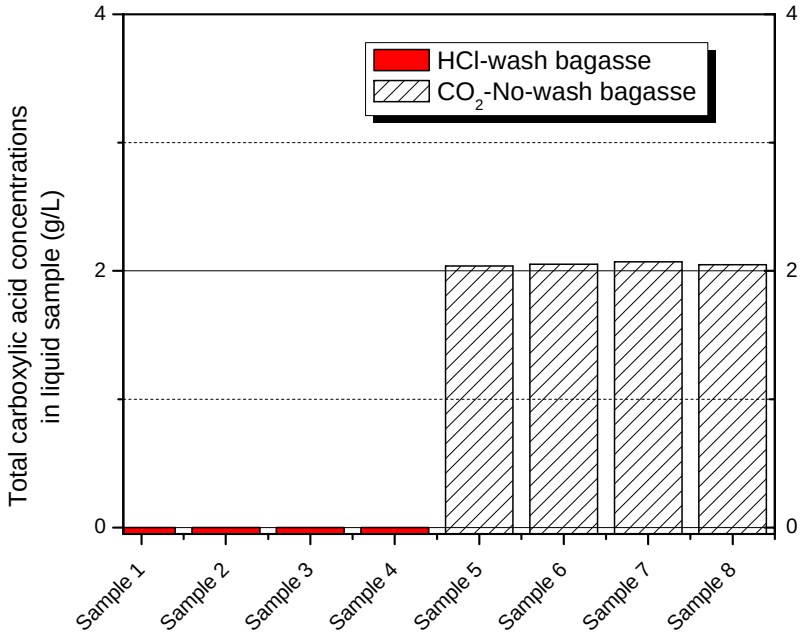


Figure 5-7. Comparison of the soluble residual carboxylate salts in the lime-treated bagasse. HCl washing procedure and CO_2-no-wash procedure were used.

Samples S5 to S8 in Table 5-4 show that there is no detectable carboxylic acid in the lime-treated bagasse if HCl washing is used. The soluble calcium salts had been fully removed by HCl washing. This also shows that the 6th loop of HCl washing (24 hours) is sufficient for removing calcium salts, because no more residual soluble calcium salts were left. This is important when the fermentation performance of different bagasse treatment is compared.

5.3.2 Mixed effects of ammonium bicarbonate and calcium carbonate

To verify the potential mixed effect of the residual calcium salts with the desired ammonium bicarbonate buffer, waste paper is a good biomass substrate. No additional lime treatment is required for paper to enhance its digestibility. Paper fed to anaerobic fermentations does not contain residual calcium salts. Therefore, investigation of a single factor of a mixed buffer consisting of ammonium bicarbonate and calcium carbonate is possible. Paper mixed with added calcium carbonate is the so-called "simulated lime-treated paper" in this section.

Table 5-5 lists the fermentation configurations used to check the mixed effects of ammonium bicarbonate and calcium carbonate on anaerobic fermentations. R1 used the original paper without additional calcium carbonate, whereas R2 used the same amount of paper but with additional calcium carbonate. The amount of calcium carbonate was 2.70 g, based on the estimated 11.9% weight ratio in Section 5.1. Other than the initial residual calcium carbonate, both fermentations were operated under identical conditions. Varying the addition of ammonium bicarbonate was the only buffer used to control both fermentations to the desired pH of 7.0 (6.97–7.03).

Table 5-5. Paper fermentation configures to check mixed buffer effects of ammonium bicarbonate and calcium carbonate.

	Composition of biomass substrate	Initial calcium carbonate addition	Buffer System	Inoculum
R1 "original paper"	16 g paper 4 g chicken manure	NO	1 g initial NH_4HCO_3 then variable NH_4HCO_3 to maintain pH around 7.0 (6.97–7.03)	20 mL inocula from previous ammonia bicarbonate thermophilic countercurrent fermentations
R2 "simulated lime-treated paper"	16 g paper 4 g chicken manure	YES, simulated with estimated 11.9% weight ratio of calcium carbonate (2.70 g $CaCO_3$)	1 g initial NH_4HCO_3 then variable NH_4HCO_3 to maintain pH around 7.0 (6.97–7.03)	20 mL inocula from previous ammonia bicarbonate thermophilic countercurrent fermentations

Total carboxylic acid concentration and pH for Fermentations R1 and R2 in Table 5-5 are shown in Figures 5-8 and 5-9. From Figure 5-9, the pH in both fermentations is well controlled around 7.0.

Figure 5-8 compares the product concentration between Fermentation R1 (original paper) and Fermentation R2 (simulated lime-treated paper). There was similar performance for both fermentations. The product concentration in Fermentation R1 is very close to that in Fermentation R2. There is no significant product concentration difference between two buffer systems. In 17 days, Fermentation R1 produced 20.33 g/L acid, whereas Fermentation R2 obtained 19.64 g/L. The acid concentration on Day 29 was 27.72 g/L and 27.06 g/L for Fermentations R1 and R2, respectively.

The similar fermentation performance between the original paper fermentations and the simulated "lime-treated" paper fermentations demonstrated that the mixed effect of ammonium bicarbonate and calcium carbonate was not an issue for ammonium bicarbonate buffered fermentations. This probably results from the solubility difference of both buffers. Ammonium bicarbonate is highly soluble in water, whereas calcium carbonate is nearly insoluble near pH 7.0. The carboxylic acids produced from anaerobic fermentation should first react with the highly soluble buffer (i.e., ammonium bicarbonate). Once the ammonium bicarbonate is consumed, the excess carboxylic acids will start to consume calcium carbonate. The consumption of calcium carbonate will be difficult if the desired pH is controlled around 7.0.

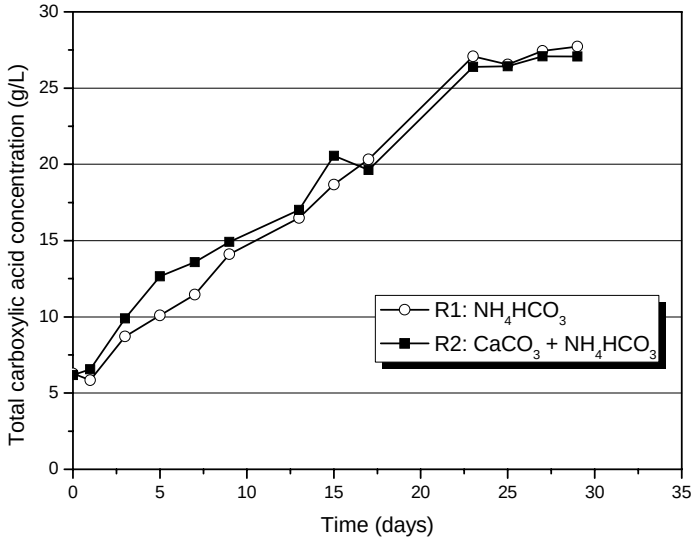


Figure 5-8. Total carboxylic acid concentration for paper fermentations used to examine effects of residual calcium salts. Ammonium bicarbonate was the pH buffer.

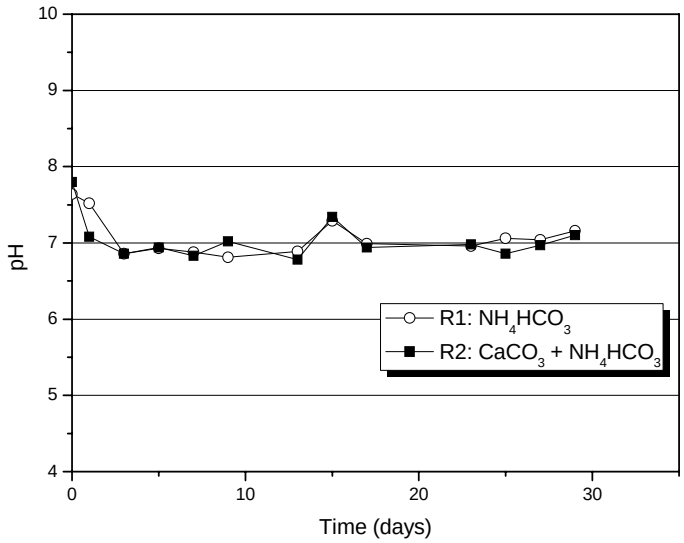


Figure 5-9. pH profiles for paper fermentations used to examine effects of residual calcium salts. Ammonium bicarbonate was the pH buffer.

5.3.3 Anaerobic fermentation of HCl-washed lime-treated bagasse

The mixed effect of ammonium bicarbonate and calcium carbonate is not significant in paper fermentations (Section 5.3.2). The lime-treated bagasse was specially washed out by HCl solution to remove the soluble calcium salts and calcium carbonate fine particles in the biomass surface. The idea is the original lime-treated bagasse (Sample F in Table 5-2) is simulated by the mixture of the HCl washed lime-treated bagasse (Sample E in Table 5-2) and the calcium salts. This section is used to check the mixed effects of both buffers in bagasse fermentations.

Table 5-6 illustrates the fermentation configurations used to check effects of residual calcium salts on ammonium bicarbonate buffered fermentations. Fermentation R3 used lime-treated bagasse with an HCl wash (Sample E in Table 5-2), whereas Fermentation R4 was for the lime-treated bagasse with CO_2 neutralization (Sample F in Table 5-2). Other than the initial bagasse, both fermentations were operated identically. Varying addition of ammonium bicarbonate was the only buffer used to control both fermentations in desired pH 7.0 (6.97–7.03).

Total acid concentrations and acetate contents for Fermentations R3 and R4 are shown in Figures 5-10 and 5-11. Figure 5-10 illustrates a similar performance for both fermentations. Both the product concentration and acetate concentration in Fermentation R3 are very close to those in Fermentation R4. In 28 days, Fermentation R3 produced 19.85 g/L total acids, whereas Fermentation R4 obtained 20.27 g/L. There was no significant product concentration difference between two buffer systems.

The similar fermentation performance between the hot-lime-water-treated bagasse and the HCl-washed lime-treated bagasse showed that the mixed effect of ammonium bicarbonate and calcium carbonate was not an important factor for ammonium bicarbonate buffered fermentations.

Table 5-6. Bagasse fermentation configures to check effects of residual calcium salts from lime-treated bagasse.

	Composition of biomass substrate	Biomass treatment methods	Buffer System	Inoculum
R3*	16 g lime-treated bagasse 4 g chicken manure	HCl neutralization w/ water washing (Sample E in Table 5-2)	1 g initial NH_4HCO_3 then variable NH_4HCO_3 to maintain pH around 7.0 (6.97–7.03)	20 mL inocula from previous ammonia bicarbonate thermophilic countercurrent fermentations
R4*	16 g lime-treated bagasse 4 g chicken manure	CO_2 neutralization w/o water washing (Sample F in Table 5-2)	1 g initial NH_4HCO_3 then variable NH_4HCO_3 to maintain pH around 7.0 (6.97–7.03)	20 mL inocula from previous ammonia bicarbonate thermophilic countercurrent fermentations

* Experiments were performed in duplicate and average results are reported.

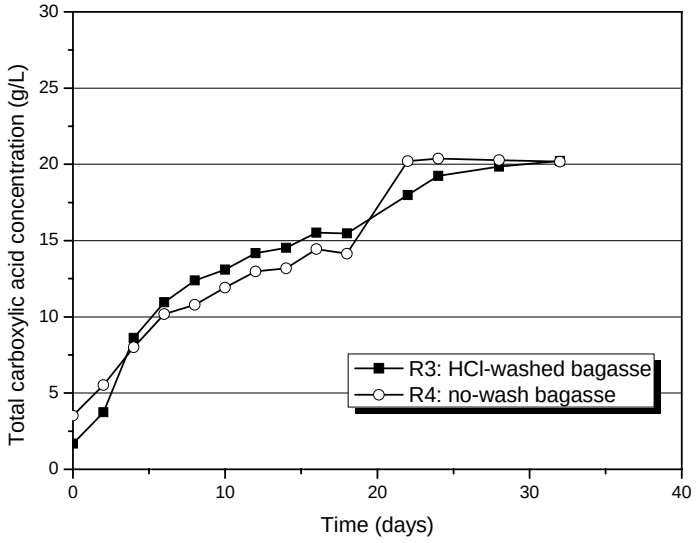


Figure 5-10. Total carboxylic acid concentration for bagasse fermentations. Ammonium bicarbonate was the buffer.

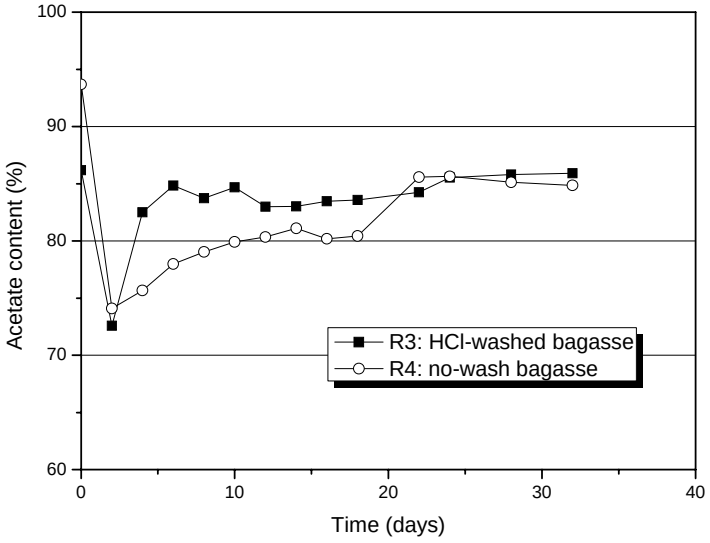


Figure 5-11. Acetate content for bagasse fermentations. Ammonium bicarbonate was the buffer.

5.3.4 Effects of biomass pretreatment on ammonium bicarbonate buffered fermentations

So far, there are three biomass treatment methods used in this dissertation:

a. hot-lime-water treatment (2 hours)
b. air-lime treatment (8 weeks)
c. ammonia solution treatment

This section is an investigation on the effects of residual calcium salts and aims to start a preliminary evaluation of effects of all three different treatment methods on the ammonium bicarbonate buffered fermentations.

Table 5-7 lists the fermentation configurations used to check the effects of treatment methods on ammonium bicarbonate buffered fermentations. Fermentation M1 used the improved long-term air-lime-treated bagasse, whereas Fermentation M2 is for the traditional hot-lime-water-treated bagasse. The air-lime-treated bagasse in Fermentation M1 was taken from Jones's long-term lime-plus-air bagasse pretreatment batch (Jones 2007) and was different from the air-lime-treated bagasse in Chapter IX. Fermentation M3 used the ammonia-treated bagasse. The total volume of each fermentation was 250 mL. The mixture of 80 wt% bagasse (16 g) and 20 wt% raw chicken manure (4 g) was the initial substrates for all fermentations in this section. Varying addition of ammonium bicarbonate was the only buffer used to control fermentations in a desired pH range (around 7.0).

Table 5-7. Fermentation configures to examine effects of different pretreatment methods on ammonium bicarbonate buffered fermentations.

	Treated bagasse	Buffer	Inoculum
M1*	16 g air-lime-treated bagasse (Jones 2007)	1 g initial NH_4HCO_3; then variable NH_4HCO_3 to maintain pH around 7.0	20 mL inocula from previous ammonia bicarbonate buffered fermentations
M2*	16 g hot-lime-water-treated bagasse	1 g initial NH_4HCO_3; then variable NH_4HCO_3 to maintain pH around 7.0	20 mL inocula from previous ammonia bicarbonate buffered fermentations
M3*	16 g ammonia-treated bagasse	1 g initial NH_4HCO_3; then variable NH_4HCO_3 to maintain pH around 7.0	20 mL inocula from previous ammonia bicarbonate buffered fermentations

* Experiments were performed in duplicate and average results are reported.

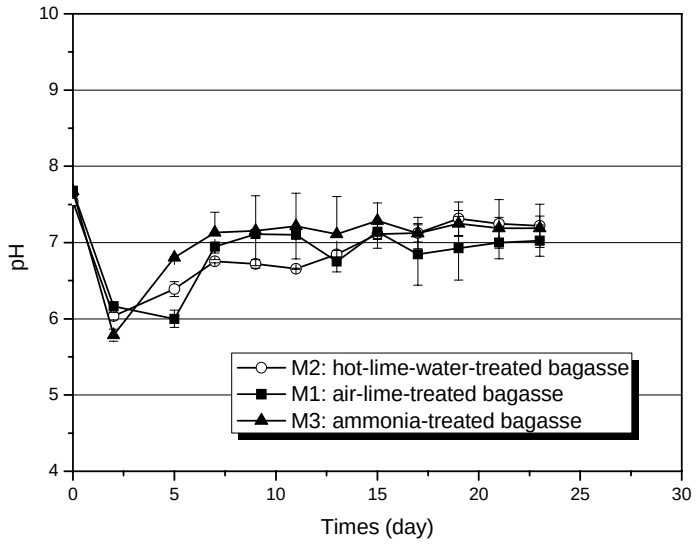


Figure 5-12. pH profiles for ammonium bicarbonate buffered fermentations using different biomass treatment methods. Error bar is for duplicate and indicates ± 1 standard deviation.

Figure 5-12 shows the pH profile for all fermentations studied in this section. In the first week, microorganisms digested the highly reactive portions of the biomass. The rapidly produced carboxylic acids reached the buffer capacity of ammonium bicarbonate and consumed most of the ammonium bicarbonate in the fermentation broth. Other than the first week, the fermentation was well controlled in the desired pH range (around 7.0).

The total carboxylic acid concentrations and acetate contents for Fermentations M1 and M2 are illustrated in Figures 5-13 and 5-14. Figure 5-13 shows that there was similar product concentration for both fermentations in the first week. Fermentation M1 (long-term air-lime-treated bagasse) exceeded Fermentation M2 (hot-lime-water-treated bagasse) in both product concentration and acetate content. In 29 days, Fermentation M1 (long-term air-lime-treated bagasse) produced 26.73 g/L, whereas Fermentation M2

(hot-lime-water-treated bagasse) obtained 16.43 g/L acids. There was a significant product concentration difference between the two treated bagasses. Long-term air-lime treatment proved to be a better treatment than the hot-lime-water treatment.

Figures 5-15 and 5-16 compare the product concentration and acetate content between Fermentation M1 (air-lime-treated bagasse) and Fermentation M3 (ammonia-treated bagasse). In 29 days, Fermentation M1 (air-lime-treated bagasse) produced 26.73 g/L, whereas Fermentation M3 (ammonia-treated bagasse) obtained 18.38 g/L acids. There were no residual calcium salts in the ammonia-treated bagasse. The air-lime-treated bagasse was neutralized by acetate acid to consume the excess lime (Jones 2007); therefore, there is little calcium salts in these air-lime-treated bagasse. Some small calcium carbonate fine particles may still stay in the biomass micropores, which is the same issue as the HCl-washed hot-lime-water-treated bagasse. The higher product concentration in Fermentations M1 (air-lime-treated bagasse) than Fermentation M3 (ammonia-treated bagasse) suggest that small calcium carbonate fine particles that may reside in the lime-treated bagasse may be not an issue to ammonium bicarbonate buffered fermentations.

Figures 5-17 and 5-18 show that ammonia treatment has comparable performance with the hot-lime-water treatment. The similar conclusion had been reported in Section 4.3 of Chapter IV. This similar fermentation performance of ammonia-treated bagasse and hot-lime-water-treated bagasse suggests that the residual calcium salt particles residing in the lime-treated biomass may not affect ammonium bicarbonate buffered fermentations.

In conclusion, as respect to fermentation performance, long-term air-lime treatment is the best treatment method for bagasse, but it takes 2 months pretreatment time. Ammonia pretreatment has comparable performance with hot-lime-water treatment. Residual calcium salts in lime-treated bagasse are not an issue for ammonium bicarbonate buffered fermentation.

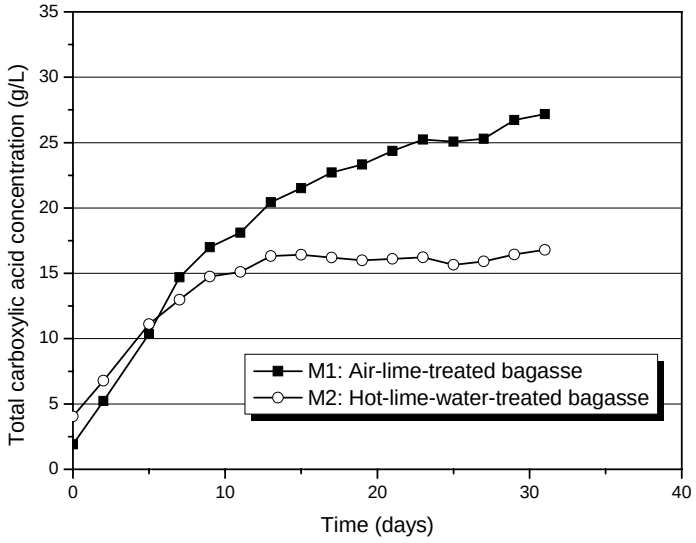


Figure 5-13. Total carboxylic acid concentration for air-lime-treated bagasse and hot-lime-water-treated bagasse.

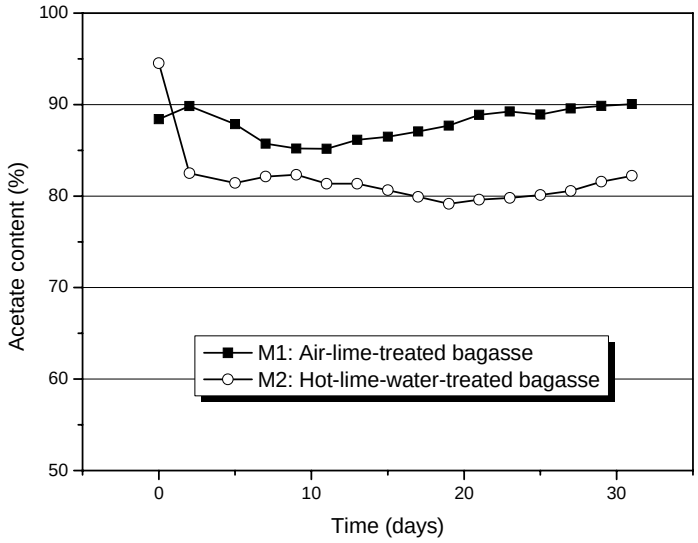


Figure 5-14. Acetate contents in fermentation product for air-lime-treated bagasse and hot-lime-water-treated bagasse.

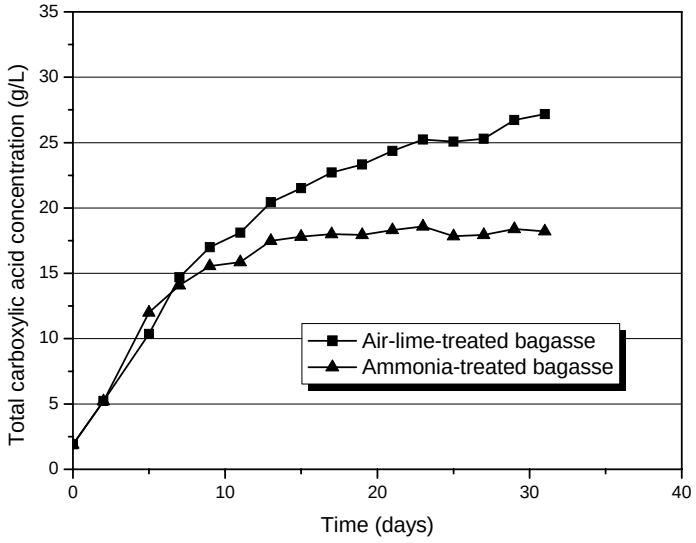


Figure 5-15. Total carboxylic acid concentration for air-lime-treated bagasse and ammonia-treated bagasse.

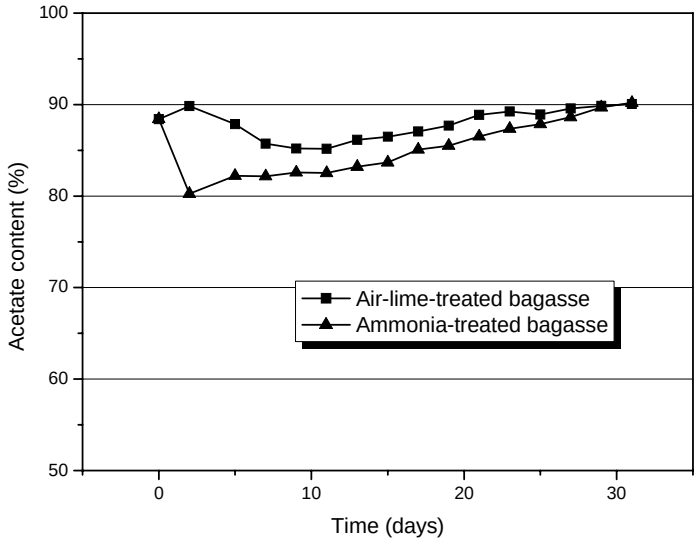


Figure 5-16. Acetate content in fermentation product for air-lime-treated bagasse and ammonia-treated bagasse.

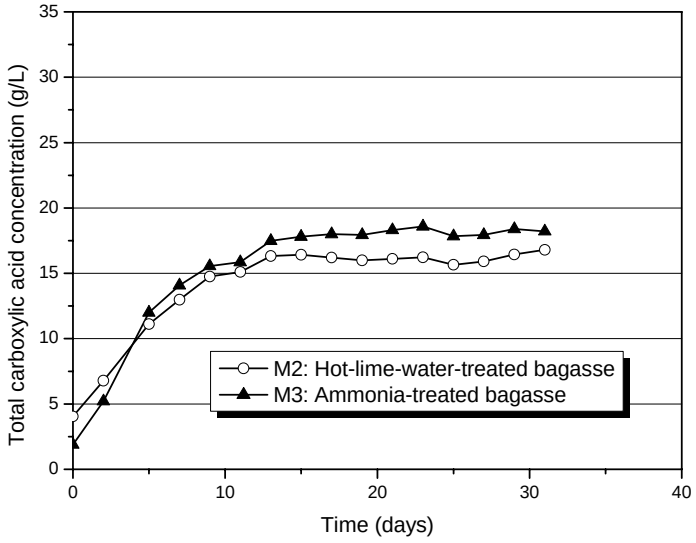


Figure 5-17. Total carboxylic acid concentration for hot-lime-water-treated bagasse and ammonia-treated bagasse.

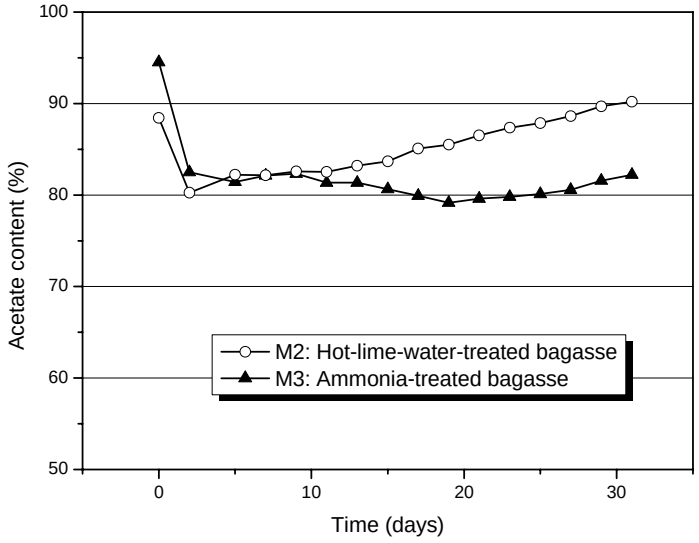


Figure 5-18. Acetate content in fermentation product for hot-lime-water-treated bagasse and ammonia-treated bagasse.

5.4 Conclusions

It has been estimated that about 11.9% (wt) of residual calcium salts remain in lime-treated biomass. This chapter focuses on examining the potential negative effect of these residual calcium salts on anaerobic fermentations buffered by ammonium bicarbonate. Furthermore, three different biomass treatments were evaluated based on fermentation performance of the treated biomass. The following conclusions are based on batch fermentations under thermophilic conditions:

1) "Simulated lime-treated paper" with additional 11.9% calcium carbonate does not exhibit significant fermentation differences from the original paper substrate. The simulated addition of calcium carbonate does not block the paper micropores and functions as pH buffer only. The mixed effect of ammonium bicarbonate and calcium carbonate does not show negative effects on further fermentations.

2) HCl neutralization and washing cannot fully remove the residual calcium salts in the lime-treated biomass. Of the total residual calcium salts (based on metal composition analysis), 13% are difficult to be removed by HCl solution and assumed to still stay in the biomass micropores. Further biomass fermentations showed that the residual calcium salts do not affect ammonium bicarbonate buffered fermentations.

3) Ammonia treatment has a comparable fermentation performance with the hot-lime-water treatment.

4) The improved lime treatment with air purging is preferred biomass treatment method. Long-term air-lime-treated bagasse achieved the best fermentation performance, but it requires a 2-month treatment time.

CHAPTER VI
EFFECT OF INOCULUM SOURCE ON ANAEROBIC FERMENTATION PERFORMANCE

The objectives of this chapter follow:

a) To verify our assumption that the high salt concentration in the Great Salt Lake, UT forces the microorganisms to be more "robust" and therefore produce more carboxylate salts than by the marine inoculum.
b) To compare different inoculum sources based on their anaerobic fermentation performance.
    1. The original (i.e., unadapted) Lake Inoculum 1 (referred as "black" lake inoculum) from the Great Salt Lake, UT.
    2. The original (i.e., unadapted) Lake Inoculum 2 (referred as "brown" lake inoculum) from the Great Salt Lake, UT.
    3. The mixed original (i.e., unadapted) inoculum of the equal amount of Lake Inoculum 1 and Lake Inoculum 2.
    4. The original (i.e., unadapted) marine inoculum from the seashore in Galveston island, TX.
    5. The adapted marine inoculum from previous ammonium bicarbonate countercurrent fermentation system.
c) To study the effect of temperature on anaerobic fermentation performance and obtain a conceptual understanding of the temperature effect. Thermophilic conditions (55°C) and mesophilic conditions (40°C) will be compared.

6.1 Introduction

The MixAlco process is well-developed and ready for commercialization. The ultimate objective of the research work here is to seek the optimum fermentation conditions at the laboratory scale and to provide valuable guidance for future scale-up. The direct goal is to improve biomass conversion and increase the carboxylic acid concentration in the fermentation broth. This chapter focuses on comparing different inoculum sources for the anaerobic fermentation.

The performance of an anaerobic fermentation is influenced by various fermentation conditions including pH, temperature, nutrient supply, and inoculum source. Selecting an inoculum source is an important step in the anaerobic fermentation, because it provides the species of microorganisms for the fermentation process. Whether the microorganisms from the inoculum source can adapt to the new environment determines the final production, yield, and stability of the fermentation process.

Extensive studies (Aiello Mazzarri 2002; Chan and Holtzapple 2003; Thanakoses 2002) on different inoculum sources were performed for the fermentation buffered by calcium carbonate ($CaCO_3$). The inoculum sources were collected from various locations and were divided into three different categories as listed in Table 6-1: (1) rumen fluid, (2) terrestrial inoculum, and (3) marine inoculum. Rumen fluid was the first-generation inoculum source tested for the anaerobic fermentation in the MixAlco process. The relatively complex process for collecting the rumen fluid and its weak performance relative to other inoculum sources makes it undesirable for the MixAlco process (Peterson 2006). Terrestrial inocula are the second-generation inoculum source. Various terrestrial inoculum sources investigated included swamp material from Bee Creek Park (College Station, Texas), the compost from a pile at Dr. Mark Holtzapple's house (College Station, Texas), and the compost from a pile in Southwood Valley Turf (College Station, Texas). In 2000, marine inocula were first introduced to the MixAlco process. Sediments from several seashore locations in Galveston Island, Texas were

Table 6-1. Inoculum sources for the anaerobic fermentation in the MixAlco process.

Category	Inoculum source	Inocula sampling location	Salinity [a] (salt concentration level) in environment	Fermentation buffer system
A	Rumen fluid	Cattle	Low, 0.1–0.3%	$CaCO_3$
B	Terrestrial inoculum	Various locations	Low, 0.1–0.3%	$CaCO_3$
C	Marine inoculum	Galveston Island, TX	high, 3.5%	$CaCO_3$ NH_4HCO_3
D	Lake inoculum	Great Salt Lake, UT	Very high, 12–25%	NH_4HCO_3

[a] Salinity is the salt concentration (by weight) in water.

collected and used as the inoculum source for the anaerobic fermentation. Terrestrial and marine inocula have been widely used in the MixAlco process.

Intensive research (Aiello Mazzarri 2002; Chan and Holtzapple 2003; Thanakoses 2002) on anaerobic fermentations buffered by calcium carbonate showed that marine inoculum is a better inoculum source compared with a terrestrial inoculum source. Thankoses (2002) found that the marine inoculum exceeded the terrestrial inoculum by increasing the total carboxylic acids concentration from 9.6 g/L to 16.2 g/L for 80% bagasse/20% chicken manure system at 40°C (mesophilic condition). Aiello Mazzarri (2002) concluded that the anaerobic fermentations using marine inoculum achieved 30% higher total carboxylic acids than that using terrestrial inoculum at 40°C (mesophilic condition). The fermentation using marine inoculum produced 26.21 g/L total carboxylic acids, whereas the fermentation using terrestrial inoculum obtained 20.66 g/L for 80% lime-treated MSW/20% SS (municipal solid wastes/sewage sludge). Chan (2002) reported a similar trend for the anaerobic fermentation buffered by calcium

carbonate at 55°C (thermophilic condition) and found that the marine inoculum achieved a higher conversion than terrestrial inoculum (0.73 vs 0.62) for long-term countercurrent fermentation using 80% corn stover/20% pig manure.

The better performance of the marine inocula than the terrestrial inocula suggested that salt concentration in the inoculum environment is a good index for finding the "ideal" inoculum source. Chan (2003) hypothesized that microorganisms from the marine source do a better job in the fermentation because they are more "robust" and better tolerate saline solutions better than terrestrial inocula. A high salt concentration in the environment leads to high extracellular osmotic pressures for the microorganisms and therefore removes water from cells via desiccation. Microorganisms from highly saline environments have adapted to the high osmotic pressure and therefore can thrive in the high salt concentration in the fermentor broth.

Recently, ammonium bicarbonate (NH_4HCO_3), a novel buffer, was introduced to the anaerobic fermentation in the MixAlco process. Using ammonium bicarbonate as a buffer, the carboxylic salt concentration in the fermentation broth can be 50%–100% higher than in fermentations using calcium carbonate ($CaCO_3$) as a buffer. The concentration increase was nearly double for 80% paper/20% chicken manure whereas it was 50–60% higher for 80% lime-treated bagasse/20% chicken manure under thermophilic conditions (e.g., 55°) in other project (***Chapter III A preliminary comparison of thermophilic fermentations using ammonium bicarbonate and calcium carbonate as a buffer***). Frank Agbogbo (2005) reported a similar doubling of total carboxylic acids for 80% paper/20% chicken manure under mesophilic conditions (e.g., 40°). The 50–100% increased salt concentration in this newly introduced ammonium bicarbonate buffered fermentation may challenge the marine inoculum even more. The highly soluble ammonium bicarbonate itself increases the salt concentration of the fermentation system when added to control pH. Furthermore, the increased carboxylate salt concentration in the fermentation broth also increased the total salt concentration.

This combined increased salt concentration (e.g., over 5% salinity) may inhibit the growth of microorganisms from the marine inoculum source, which was adapted to 3.5% salinity. It will be rational and promising to seek an inoculum source that contains more "robust" microorganisms able to handle higher salt concentrations than the marine inoculum, and thus may be better able to adapt to the ammonium bicarbonate fermentation.

The Great Salt Lake (GSL) in Utah State is a good choice (Morgan 1947). It is the largest U.S. Lake and the 4th largest terminal lake in the world. The salinity of the Great Salt Lake is 12–25%, which is 3 to 5 times higher than that of the ocean (i.e., 3.5%). Based on the success of the marine inoculum in the calcium carbonate buffered fermentation, the lake inocula from the Great Salt Lake was hypothesized to be a "better" inoculum source than the marine inocula because it may contain more "robust" microorganisms that can survive in a high-salinity environment. Indeed, one of the objectives of this project was to verify this assumption.

In summary, the study in this chapter was undertaken to investigate the feasibility of using the lake inoculum from the Great Salt Lake, UT for the anaerobic fermentation in the MixAlco process. The effect of temperature on the fermentation performance was also assessed. Both thermophilic conditions (55°C) and mesophilic conditions (40°C) were evaluated to compare different fermentation sources: marine inoculum and salt lake inoculum.

6.2 Methods and materials

Table 6-2 summarizes the pretreatment and fermentation conditions used in this project.

6.2.1 Selection of biomass feedstock

Sugarcane bagasse from the Lower Rio Grande Valley (LRGV), Texas and chicken manure from the Department of Poultry Science at Texas A&M University, Texas were used as the biomass feedstock. Bagasse was the carbon source of the fermentation whereas chicken manure was the nutrient source. The fresh bagasse was dried, ground, and passed through a 10-mesh sieve. The milled bagasse was pretreated by lime at 100°C for 2 hours followed by carbon dioxide neutralization and drying in an oven for another 2 days. The average volatile solids content for the raw chicken manure was 74.36% and the average volatile solids content for the lime-treated bagasse was 83.79%. The mixture of 80% (dry weight) lime-treated bagasse and 20% (dry weight) raw chicken manure was the initial substrate for the fermentations in this chapter.

6.2.2 Selection of inoculum source (sources of microorganisms)

Marine and salt lake inocula were the only two sources selected for this project. They both contain microorganisms that can resist high salt concentrations but the environmental salinity was different. The adapted marine inoculum from the previous NH_4HCO_3 countercurrent thermophilic fermentations was used as an "internal standard" to establish a "possible and reasonable" performance standard for the other fermentation systems with the different original (i.e., unadapted) inoculum sources.

The original (i.e., unadapted) inoculum was sampled and prepared as follows:

Table 6-2. Matrix table for inoculum source comparison.

Operating conditions		Case	Used
Substrate (nutrient source)		Chicken manure	√
Substrate (carbon source)		Bagasse	√
		Paper	
Pretreatment (Bagasse)	Chemical	Lime solid, $Ca(OH)_2$	√
		Aqueous ammonia, $NH_3 + H_2O$	
	Temperature	55°C	
		100°C	√
		Room temperature (20–25°C)	
	Time	2 hours	√
		1 day	
		12 days	
		1 month	
	Neutralization	Carbon dioxide, CO_2	√
		Hydrogen chloride, HCl	
		Acetic acid, CH_3COOH	
		D.I. water washing, no chemicals	
	Drying method	105°C Oven (2 d)	√
		Room temperature hood (2 d)	
Fermentation	Temperature	Thermophilic conditions (55°C)	√
		Mesophilic conditions (40°C)	√
	Neutralization buffer	Ammonium bicarbonate, NH_4HCO_3	√
		Calcium carbonate, $CaCO_3$	
	Methane inhibitor	Iodoform	√
	Inoculum source	Original (unadapted) marine inoculum	√
		Adapted marine inoculum from previous countercurrent fermentation	√
		Original (unadapted) lake inoculum	√

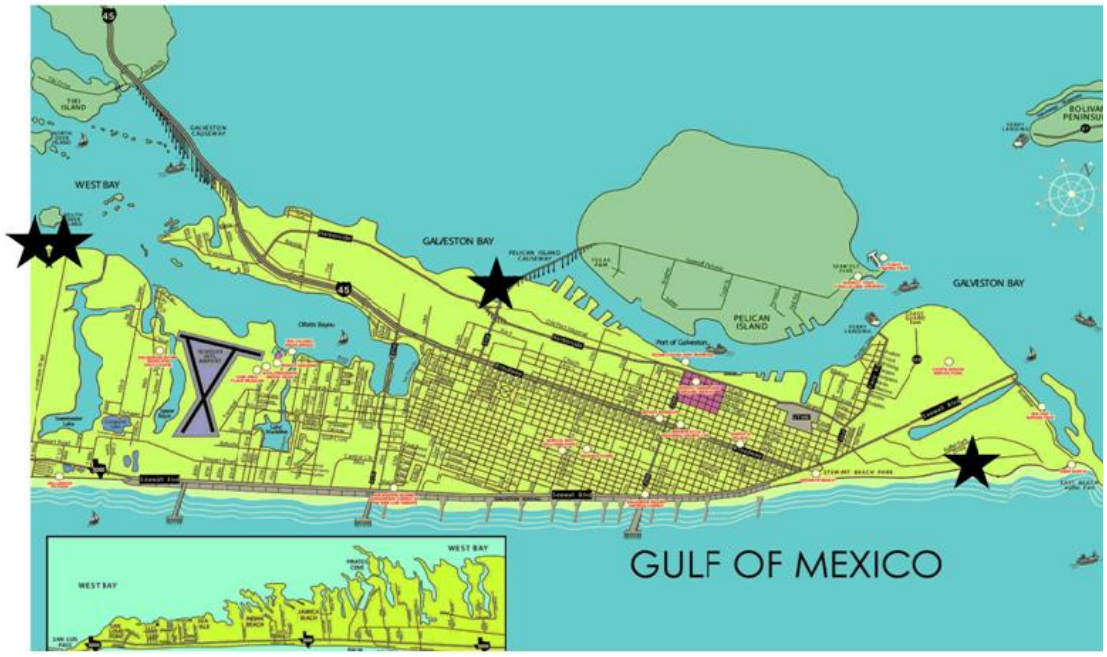


Figure 6-1. Sampling locations for marine inoculum from Galveston Island, TX. The black stars indicate sample locations for the marine inocula.

Source A: Marine Inoculum from Galveston Island, Texas

Sediment from Galveston Island (Galveston, Texas) shores was used as the fermentation inoculum source in this project and is described as "original marine inoculum." As illustrated in Figure 6-1, four marine inoculum samples were taken from different places, one from East beach (Apffel Park), one from Harborside & 51st, and two from Sportman's road. The sediment samples were taken from 0.5-m-deep holes, and stored in bottles filled with anaerobic liquid medium (i.e., deoxygenated water). Equal amounts of sediment liquid from each bottle were mixed and used as fermentation inocula.

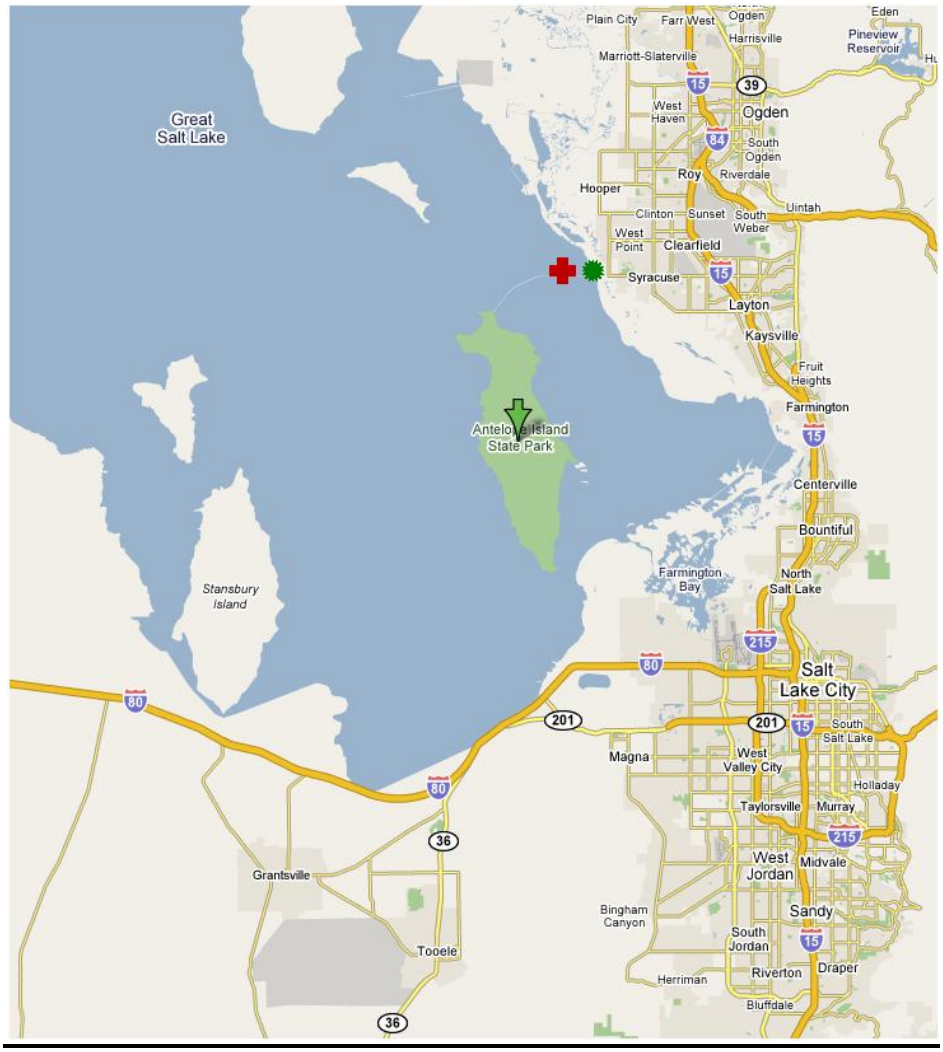


Figure 6-2. Sampling locations for Salt Lake inoculum from the Great Salt Lake, UT. The red cross indicates sample location for "black lake inocula." The green starbust indicates sample location for "brown lake inocula."

Source B: Lake Inoculum from the Great Salt Lake, Utah

Sediment from the lakeside area of the Great Salt Lake (Salt lake city, Utah) were used as the fermentation inoculum source in this project and is described as "original lake inoculum." As shown in Figure 6-2, the salt lake inocula were collected from two different locations, and are labeled as "black" and "brown" based on the sample color. The lake inoculum samples were placed in 1-L centrifuge bottles filled with

deoxygenated water and kept in the freezer once they were delivered to our laboratory. The defrosted liquid was fully mixed and centrifuged for 20 minutes at 4,000 rpm. The supernant was used as the inoculum for the anaerobic fermentations.

Extensive studies have been performed previously for the marine inoculum sources in the anaerobic fermentations in the MixAlco process, whereas this is the first time salt lake inoculum has been studied. More attention was paid to the salt lake inocula sources in this project. Both the "brown" lake inoculum and the "black" lake inoculum were studied at 40°C and 55°C. A mixture of equal amounts of the "brown" lake inoculum and the "black" lake inoculum were further examined at 55°C because the thermophilic fermentation is the major topic in this dissertation.

6.2.3 Buffer selection

Ammonium bicarbonate (NH_4HCO_3) was used as the only buffer system in this project. As mentioned before, the previous results showed that ammonium bicarbonate is a preferred buffer for the anaerobic fermentation in the MixAlco process. The current research interest is focused on optimizing the ammonium bicarbonate fermentation. Calcium carbonate ($CaCO_3$) was not selected as a buffer to optimize the performance in this project. The selected inoculum sources were compared based on the performance of the fermentations buffered by ammonium bicarbonate.

6.2.4 Batch fermentation

Other than countercurrent transfer fermentation, batch fermentation was used in this chapter. The batch fermentation procedures are detailed in Chapter II. The liquid volume in all fermentations was 250 mL. The temperature was maintained at 55°C (thermophilic condition) or 40°C (mesophilic condition). The substrate, 20 g of 80% lime-treated bagasse/20% raw chicken manure, was the initial biomass feedstock for the batch fermentations. Table 6-3 lists the fermentation configurations used in this chapter. All of the batch fermentations were started at the same time and operated under identical conditions.

Table 6-3. Experimental condition matrix for anaerobic fermentation using different inoculum sources.

Configuration		Biomass feedstock		Inoculum source	Fermentation temperature (°C)	Iodoform (mg/(L·day))	Nutrient mixtures (g/(L·day))
		Lime-treated bagasse (g)	Chicken manure (g)				
1	MS1–2	16	4	Original "black" lake inoculum from Great Salt Lake, UT	55	4.8	0.2
2	MS3–4	16	4	Original "brown" lake inoculum from Great Salt Lake, UT	55	4.8	0.2
3	MS5–6	16	4	Mixture of 50% of "black" lake inoculum and "brown" lake inoculum	55	4.8	0.2
4	MS7	16	4	Original marine inoculum from four shore locations in Galveston Island, TX	55	4.8	0.2
5	MS9–10	16	4	Adapted marine inoculum from previous NH_4HCO_3 countercurrent fermentation	55	4.8	0.2
6	CS1–2	16	4	Original "black" lake inoculum from Great Salt Lake, UT	40	4.8	0.2
7	CS3	16	4	Original marine inoculum from four shore locations in Galveston Island, TX	40	4.8	0.2
8	CS4	16	4	Adapted marine inoculum from previous NH_4HCO_3 countercurrent fermentation	40	4.8	0.2
9	CS5	16	4	Original "brown" lake inoculum from Great Salt Lake, UT	40	4.8	0.2

The pH in all batch fermentations was controlled around 7.0 (i.e., 6.97–7.03). If the measured pH fell down 7.0, ammonium bicarbonate was continuously added to the fermentor until the pH reached the preset range (6.97–7.03). No additional ammonium bicarbonate was required if the pH was above 7.0. The carboxylic acids produced by the microorganisms could lower pH and somewhat adjusted pH themselves.

Nutrients and methane inhibitor concentrations are environmental factors that can influence the growth of the culture and may be a limiting factor for the entire fermentation performance. Chicken manure was the nutrient substrate source and supplied most of the required nutrients for the microorganisms in the fermentation. Additional nutrients mixture could be used to fully eliminate the nutrient effect. Furthermore, iodoform, a methane inhibitor, was added to reduce the effect of possible methanogenesis. The addition of a nutrient mixture and iodoform ensured that the "best" possible fermentation performance is compare based on the different inoculum sources only. Nutrient mixture and iodoform (methane inhibitor) were added to each fermentation at ratio of 0.2 g/(L·day) and 4.8 mg/(L·day), respectively. Both quantities were shown to be adequate for the growth of the microorganisms in the countercurrent fermentation using ammonium bicarbonate under thermophilic condition.

6.2.5 Data analysis

The total carboxylic acid concentration, conversion, selectivity, and yield were used to compare the different fermentation performance using different inoculum sources. In general, higher conversion, higher yield, and higher selectivity are desired. The following equations were applied in this chapter:

$$\text{conversion} = \frac{\text{digested VS}}{\text{initial VS feed}}$$

$$\text{yield} = \frac{\text{total acids produced}}{\text{initial VS feed}}$$

$$\text{selectivity} = \frac{\text{total acids produced}}{\text{digested VS}}$$

6.3 Results and discussions

6.3.1 pH and gas production

pH plays a very important role in the anaerobic fermentation. For every microorganism, there is a particular pH where its activity is maximal. The mixed culture of microorganisms in the ammonium bicarbonate buffered fermentation system is sensitive to pH changes, as shown in Chapter III. Most microorganisms grow best under neutral pH conditions (i.e., 7.0), because other pH may adversely affect metabolism by altering the chemical equilibrium of enzymatic reactions, or by actually destroying the enzymes. Therefore, the desired pH for our fermentation was selected as 7.0 (6.97–7.03). Ammonium bicarbonate (NH_4HCO_3) was used as a buffer to maintain the desired pH environment for the microorganisms. No additional ammonium bicarbonate was required if the pH was above 7.0.

Figures 6-3 and 6-4 show the pH profile of the mesophilic fermentations, whereas Figures 6-5 and 6-6 exhibit the pH profile of the thermophilic fermentations. The pHs reported in those figures were measured when the fermentors were opened under nitrogen purging, which was used to keep the batch fermentations under anaerobic condition. In general, the required addition of ammonia bicarbonate to the fermentation system has a positive relationship with the carboxylic acids produced by the microorganisms.

Depending on the pH, the anaerobic fermentation has two stages:

(1) pH unstable period: There was obvious pH turbulence in the first 10 days for all batch fermentations investigated. Large amounts of NH_4HCO_3 were required to adjust the pH to the desired range. The microorganisms consumed the “easy-to-digest” portions of the biomass during this period, and rapidly produced carboxylic acids, which exceeded the pH buffer capacity of the added ammonium bicarbonate.

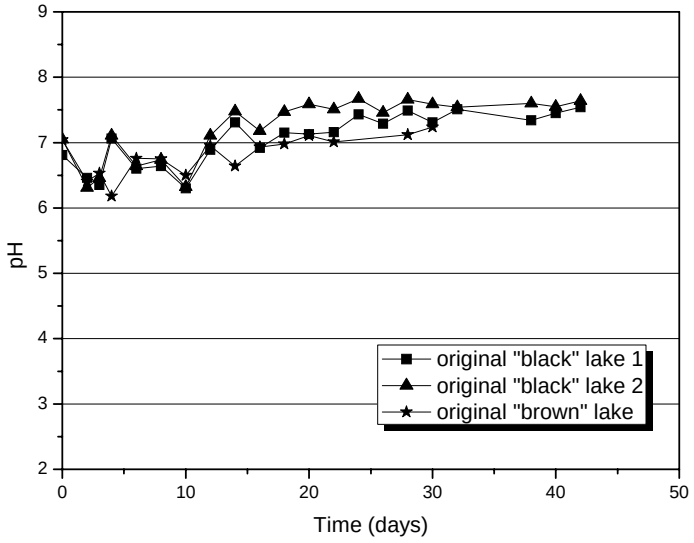


Figure 6-3. pH pattern during the fermentation of 80% lime-treated bagasse/20% chicken manure using different lake inocula under mesophilic condition (40°C).

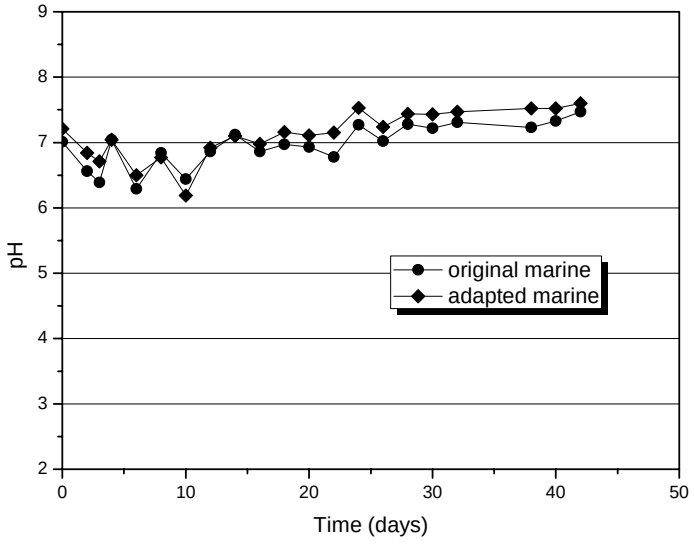


Figure 6-4. pH pattern during the fermentation of 80% lime-treated bagasse/20% chicken manure using different marine inocula under mesophilic condition (40°C).

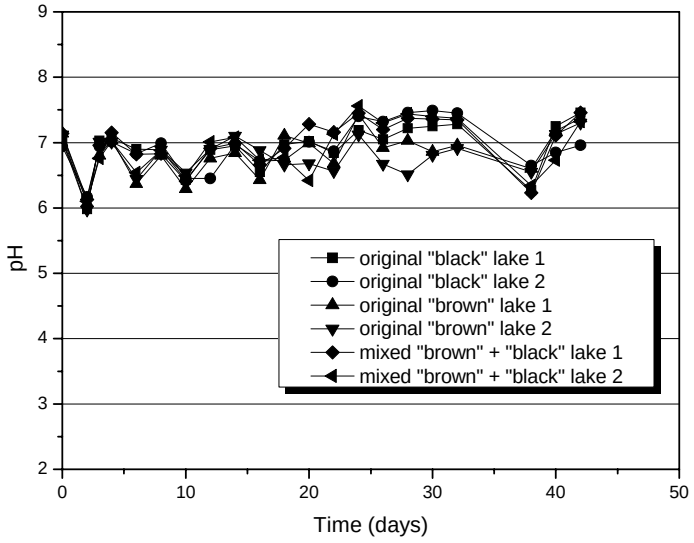


Figure 6-5. pH pattern during the fermentation of 80% lime-treated bagasse/20% chicken manure using different lake inocula under thermophilic condition (55°C).

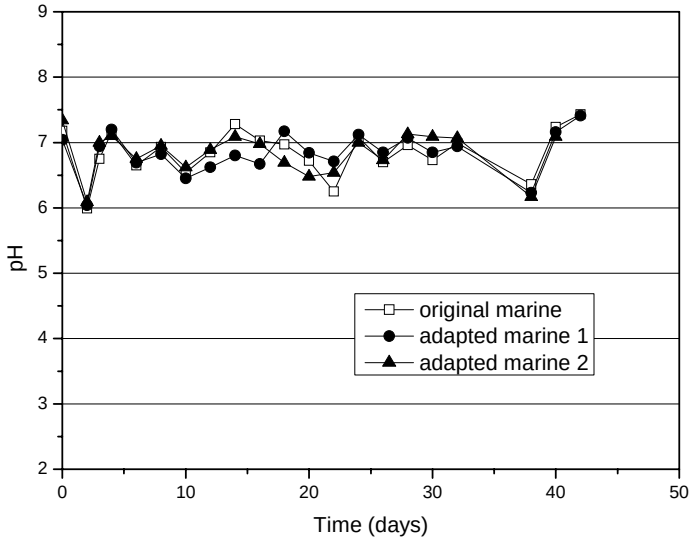


Figure 6-6. pH pattern during the fermentation of 80% lime-treated bagasse/20% chicken manure using different marine inocula under thermophilic condition (55°C).

(2) pH stable period: The fermentation reaction was relatively slow during this period. Very little NH_4HCO_3 was required to maintain the pH around 7.0. The microorganisms mainly digested the "hard-to-digest" portions of the biomass because the "easy-to-digest" portions were nearly consumed already.

As illustrated in Figures 6-7 and 6-8, the typical gas detected by GC is nitrogen (N_2), carbon dioxide (CO_2), and possible methane (CH_4). Although there was hydrogen (i.e., H_2) and other possible gases produced by anaerobic fermentations in the same time, those gases are not a concern in this chapter. Methane and carbon dioxide were the monitored gases in this chapter. Nitrogen is a carrier gas used to keep the fermentation system anaerobic condition and not the fermentation product. Abiotic carbon dioxide (CO_2) is produced by neutralizing the buffer ammonium bicarbonate and the produced carboxylic acids from the anaerobic fermentation.

$$NH_4HCO_3 + CH_3(CH_2)_xCOOH \leftrightarrows CH_3(CH_2)_xCOONH_4 + H_2O + CO_2$$

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

Biotic CO_2, another source of carbon dioxide produced in the anaerobic fermentation, was the metabolic product of the microorganisms. The total gas volume produced by the fermentation was related to the total produced carboxylic acids. The faster the carboxylic acids concentration was produced, the larger the gas volume obtained at sampling. Methane should be inhibited as much as possible because the desired carboxylic acids are the direct feedstock for the methanogens to produce methane, and therefore reduce the desired total carboxylic acids production in fermentation.

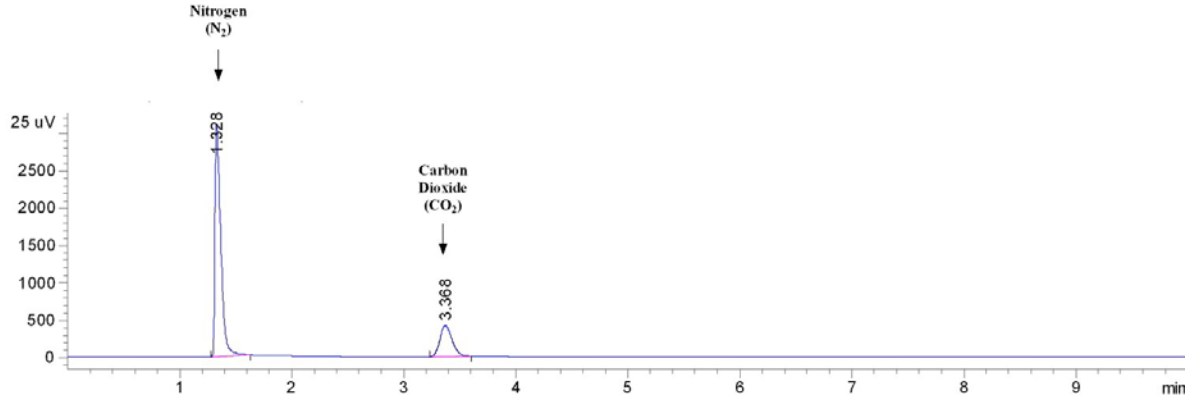


Figure 6-7. The gas composition of the batch fermentation using the original marine inoculum at 55°C on the 10th day. The detected gas composition by Agilent GC (model: 9600A+) was 77.994% nitrogen and 22.006% carbon dioxide.

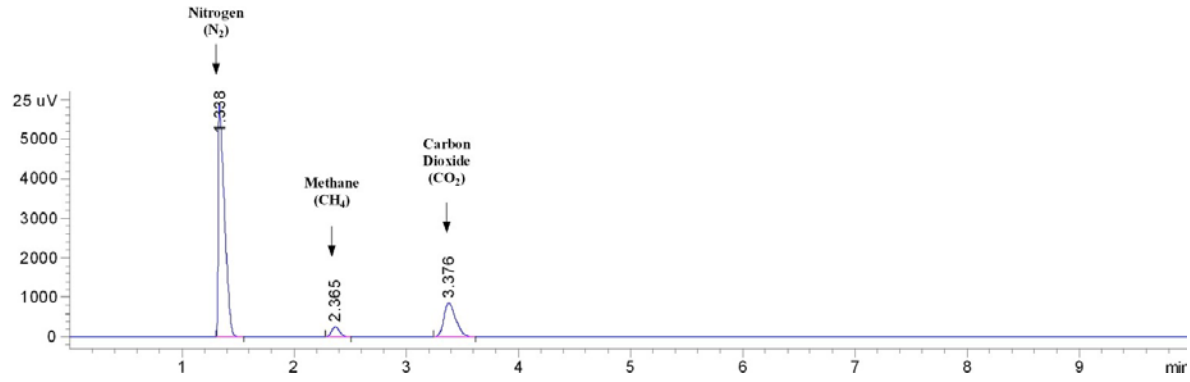


Figure 6-8. The gas composition of batch fermentation using the original "black" lake inoculum at 55°C on the 10th day. The detected gas composition by Agilent GC (model: 9600A+) was 75.099% nitrogen, 20.92% carbon dioxide and 3.98% methane.

Figures 6-9 and 6-10 show the gas production for mesophilic and thermophilic fermentations, respectively. The produced gas peaked in the first 10 days for both thermophilic and mesophilic conditions. After the first 10 days, the gas production was relatively smooth and smaller.

In summary, the first 10 days are the most important period for the anaerobic fermentation using ammonium bicarbonate buffer. More attention must be paid to the ammonium bicarbonate addition and the gas release in this period. The rapid carboxylic acid accumulation in this period overcome the pH buffer capacity of the added ammonium bicarbonate, which led to pH turbulence in the fermentation. Furthermore, rapid carboxylic acid accumulation increased the total gas production (i.e., volume) due to their reaction with ammonium bicarbonate. If the gas was not released in time, the pressure inside the fermentor could exceed the fermentor pressure limit and cause "fermentor explosion." The direct result of this possible "fermentor explosion" is the fermentor broth leakage and failure of the entire fermentation.

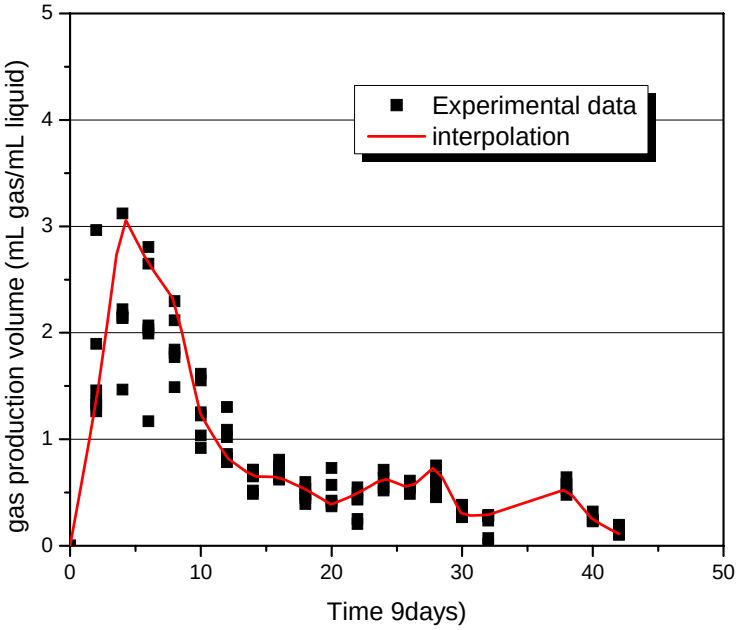


Figure 6-9. Gas production as a function of time for 80% lime-treated bagasse/20% chicken manure using different inocula under mesophilic conditions (40°C).

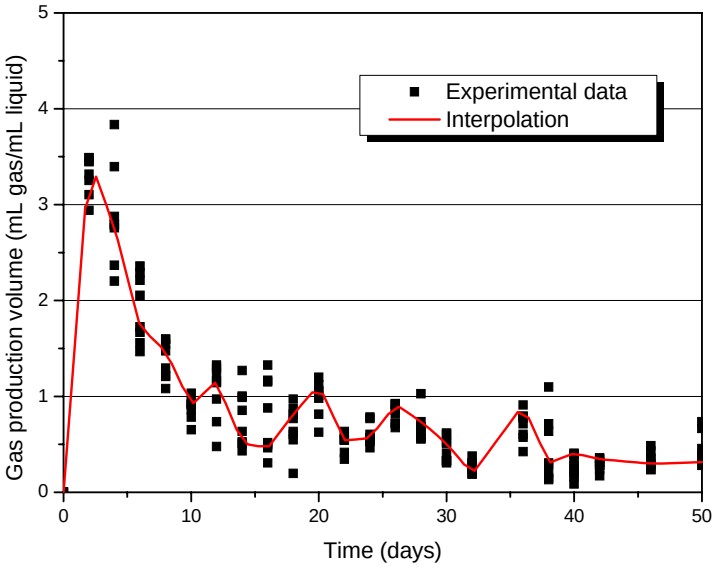


Figure 6-10. Gas production as a function of time for 80% lime-treated bagasse/20% chicken manure using different inocula under thermophilic conditions (55°C).

Table 6-4. Methane composition of gas production from anaerobic fermentations.

Inoculum sources / Temperature	Lake inoculum	Marine inoculum
40°C	ND [a]	ND
55°C	3–5%	ND

[a] ND denotes no methane detected by GC.

Table 6-4 presented the methane composition of the gas product for the fermentation with the methane inhibitor (iodoform) addition ratio of 4.8 mg/(L·day). No methane was detected during the experiments at 40°C (mesophilic condition) for all fermentations. There was 3–5% of methane production detected for all six fermentations inoculated with the original lake inoculum sources at 55°C (thermophilic condition), whereas no methane was produced in the marine inoculum fermentation at 55°C (thermophilic condition). Double-dosed methane inhibitor was added to all fermentation systems on Day 10 due to methane detected in the original lake inoculum fermentation, as shown in Figure 6-8. No further double-dose methane inhibitor was added to all fermentations because this study is not focused on investigating how to completely inhibit the methane production for the lake inoculum fermentations. The methane was not inhibited and continuously detected 3–5% in all six fermentations inoculated from the lake inoculum at 55°C. Therefore, we can safely conclude that methanologenis was not fully inhibited at 55°C for the original lake inocula with 4.8 mg/(L·day) methane inhibitor addition.

The identical addition amount of methane inhibitor (i.e., iodoform) was confirmed to be adequate in a long-term fermentation, which used identical mixture of the lime-treated bagasse and chicken manure. No methane was ever detected in that countercurrent fermentation using ammonium bicarbonate as buffer during several

months of operation time. Compared to the no methane production in the countercurrent fermentation (i.e., long-term fermentation), the batch fermentation (i.e., short-term fermentation) using lake inocula produced 3–5% methane at 55°C. The mixed culture in the lake inocula source could have a higher methane producing ability compared to the marine inocula. The more methane produced in the fermentation, the less carboxylic acid will be obtained in the anaerobic fermentation; therefore, methane is not a desired product in the anaerobic fermentations in MixAlco process. Future investigation on the lake inocula source could be focused on the selection of the methane inhibitor and its required addition rate.

6.3.2 Effect of inoculum sources on fermentation performance

The microorganisms in the anaerobic fermentation produced a very wide spectrum of carboxylic acids including acetic, propionic, butyric, valeric, caproic, and heptanoic acids. Maximizing the total acid concentration is the first task when we seek a new inoculum source. Because ammonium bicarbonate is added as a buffer to control pH in this anaerobic fermentation, ammonium carboxylate salts are obtained. The acetic acid percentage in the fermentation products was of interest also. Because acetic acid is an intermediate product to produce ethanol by esterification and hydrogenation in the MixAlco process, higher acetic acid percentages in the fermentation broth are preferred if ethanol is the desired product. Therefore, both the total carboxylic acids concentration and the acetic acid percentage were monitored to compare different inoculum sources in this section.

When a new inoculum is introduced to the fermentation system, growth of the new microorganisms in the new environment does not occur immediately. In general, this period is called the *lag phase* of the fermentation and may take several hours or several days. No significant acid production happens for most of the anaerobic fermentation

during this period. Following the *lag phase*, the growth rate of the organisms steadily increases during the so-called *exponential phase* of the fermentation. Once the substrates are nearly consumed, the growth of the microorganisms will start to slow down and may cease finally, when the culture enters the *stationary phase*. The selected inoculum source has the greatest impact on the *exponential phase,* so our focus is on the fermentation behavior in this *exponential phase*.

The different fermentation performances under mesophilic and thermophilic conditions are discussed in the following subsections:

Effect of inoculum sources on mesophilic fermentation (40°C)

The batch fermentative activities of four different inoculum sources were compared under mesophilic conditions. The inoculum source subjects are the original "black" lake inoculum, the original "brown" lake inoculum, the original marine inoculum, and adapted marine inoculum from previous countercurrent fermentations. The total carboxylic acid concentration, acetic acid percentage, VS conversion, yield, and selectivity of the fermentation were compared to evaluate the different fermentation performance of each inocula source.

a) Effect on total acids concentration

Figures 6-11 and 6-12 showed the total carboxylic acids concentration profiles for the two different inocula sources. The original "brown" inoculum seems to be the "best" of the entire four inoculum sources under mesophilic conditions (40°C). The highest acid concentration obtained for the "brown" lake inocula system was 22.3 g/L. The acid production was based on the net acid accumulation during the fermentation. The produced total acids were 19.6 g/L for the "brown" lake inoculum system compared with 13.4 g/L and 15.0 g/L produced total acids from the original marine inoculum and the adapted marine inoculum, respectively. The adapted marine inoculum obtained similar concentrations of total acids as the original marine inoculum.

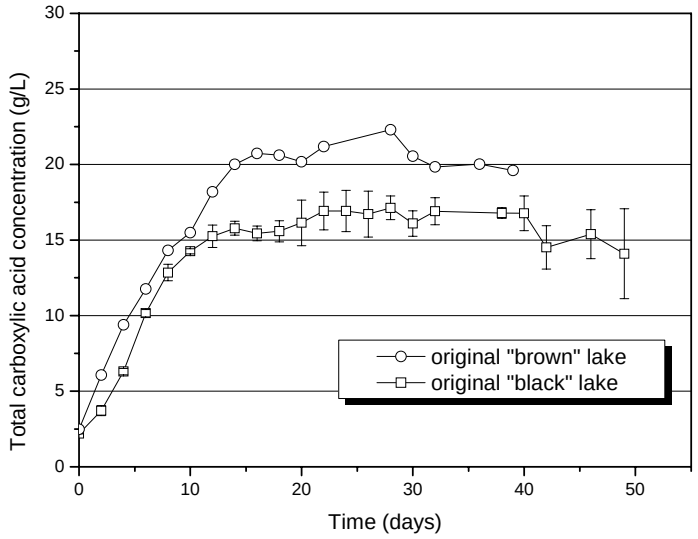


Figure 6-11. Comparison of the total acid concentration for lake inoculum source fermentations with 80 g/L of 80% lime treated bagasse/20% chicken manure under mesophilic conditions (40°C). Error bar is for duplicate and indicates 1 standard deviation.

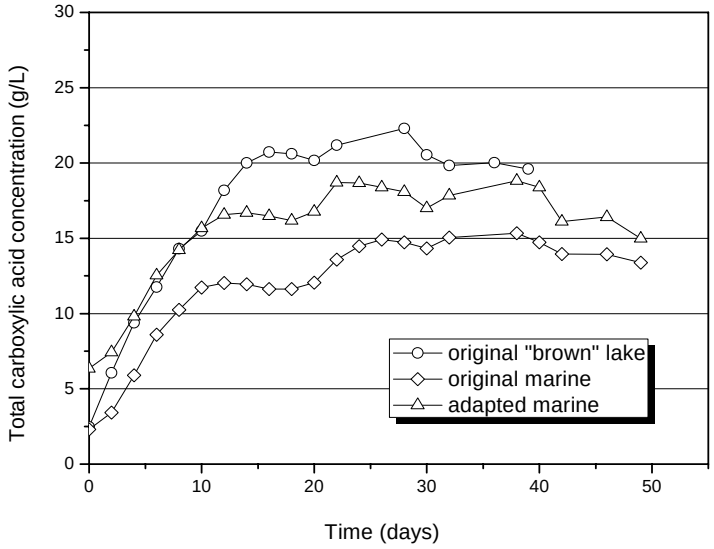


Figure 6-12. Comparison of the total acid concentration for different inoculum sources fermentations with 80 g/L of 80% lime treated bagasse/20% chicken manure under mesophilic conditions (40°C).

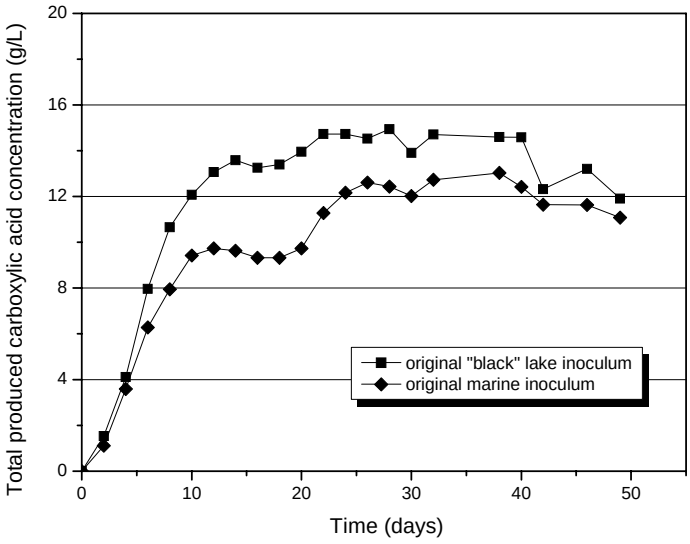


Figure 6-13. Comparison of the produced total carboxylic acids using marine inoculum and lake inoculum for 80 g/L 80% lime-treated bagasse/20% chicken manure at 40°C.

Figure 6-13 demonstrates that higher total carboxylic acid concentrations are obtained from fermentations inoculated from salt lake inoculum sources than from marine inocula sources under mesophilic conditions. For example, on Day 12, the acid concentration for the original salt lake inocula fermentation averaged 13.1 g/L whereas the acid concentration for the marine inocula fermentation averaged 10.0 g/L, a 31.1% increase. In conclusion, the original salt lake inocula had better performance in producing total carboxylic acids than the marine inocula at 40°C. In the first 3 weeks, it produced about 30% more total carboxylic acids than the marine inocula in the first 3 weeks and around 15% thereafter.

b) Effect on acetic acid production

Acetic acid is the major component in the carboxylic acids produced by the anaerobic fermentation using ammonium bicarbonate. As discussed before, a higher acetic acid percentage is preferred if the desired product is ethanol.

Figure 6-14 illustrates that the four different inoculum sources had different acetic acid selectivities under mesophilic conditions. The acetic acid content was 80–85% for the salt lake inocula system. The original "black" lake inocula had slightly higher acetic acid selectivity than the original "brown" inocula. The overall performance of the lake inocula exceeded that of the marine inocula regarding the acetic acid percentages, although they were pretty close in the first 10 days (near 80%). The original marine inocula did not have a higher acetic acid content in this study. It dropped to around 60% after 3 weeks, which was the lowest among all of the different inoculum sources regarding the acetic acid percentage.

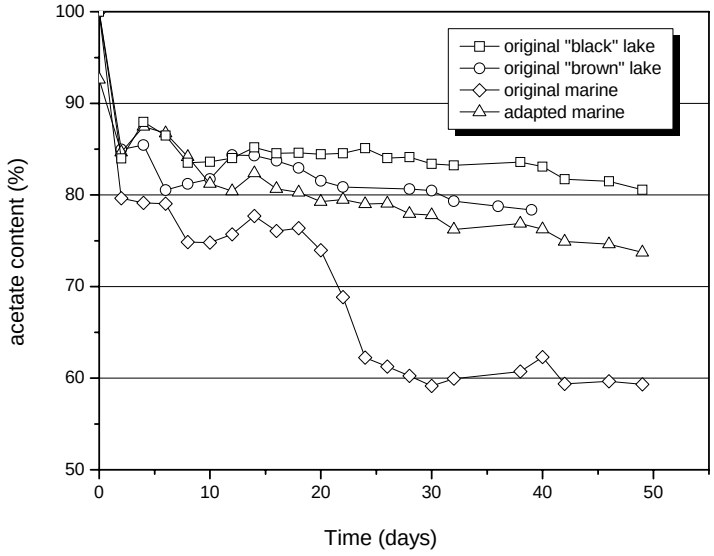


Figure 6-14. Comparison of acetic acid (C2) percentage of fermentations inoculated from different inoculum sources with 80 g/L of 80% lime treated bagasse/20% chicken manure under mesophilic condition (40°C).

c) Summary of mesophilic fermentations

Table 6-5 summarizes the fermentation results for the mesophilic fermentations. The fermentations using the salt lake inocula have a higher VS conversion, higher yield, and higher selectivity than fermentations using the marine inocula. This also shows that the lake inocula had better fermentation performance than the marine inocula under mesophilic conditions.

Table 6-5. Effect of inoculum sources on mesophilic fermentations.

Inoculum source	Peak acid concentration (g/L)	Peak acid production* (g/L)	Final acids Concentration (g/L)	VS conversion (g/g)	Yield (g acids/g VS)	Selectivity (g acids/g VS)
Original Black lake	17.23 ± 0.93	15.04 ± 0.92	14.10 ± 2.97	0.60 ± 0.03	0.24 ± 0.02	0.40 ± 0.05
Original Brown lake	22.30	19.81	19.60	0.60	0.27	0.44
Original marine	15.33	13.03	13.39	0.57	0.21	0.37
Adapted marine	18.82	12.46	14.99	0.58	0.20	0.34

* acid production = measured acid concentration – initial acid concentration
Error bar (±) indicates 1 standard deviation.

Effect of inoculum sources on thermophilic fermentations (55°C)

In this study, we focused on different salt lake inocula under thermophilic conditions. The selected lake inoculum sources were the original "black" lake inoculum, the original "brown" lake inoculum, and the mixed lake inoculum with 50:50 of "black" and "brown" lake inoculum.

The batch fermentative activities of five different inoculum sources were compared under mesophilic conditions. The inoculum sources included the three lake inoculum configurations, the original marine inoculum, and the adapted marine inoculum from previous countercurrent fermentation. The total carboxylic acid concentration, the acetic acid percentage, VS conversion, yield, and selectivity of the fermentation were compared to evaluate the different fermentation performances using the five selected inocula sources.

a) Effect on total acids concentration

Figures 6-15 and 6-16 show the total carboxylic acids concentration profiles for the three different inocula sources at 55°C (i.e., thermophilic conditions). There is no obvious difference in the total acid concentrations among all of the three selected lake inoculum sources in the first 3 weeks. After 3 weeks, the original "brown" lake source and the mixed lake source showed slight advantages. The peak total acid concentration for the mixed lake inoculum, the original "brown" lake inoculum, and the original "black" lake inoculum was 23.3 g/L, 21.6 g/L and 19.6 g/L, respectively. There was no significant difference between the marine inoculum and the salt lake sources based on the total acid concentration.

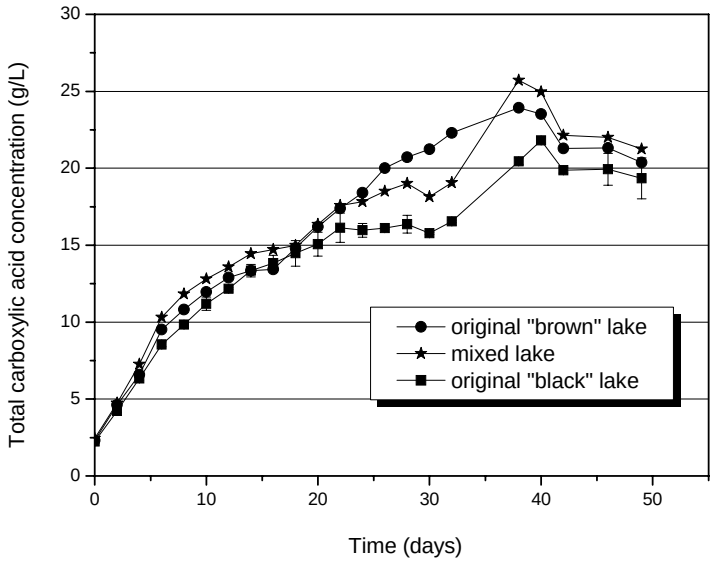


Figure 6-15. Comparison of the total acid concentration of fermentations inoculated from different lake inoculum sources with 80 g/L of 80% lime treated bagasse/20% chicken manure under thermophilic condition (55°C). Error bar is for duplicate and indicates 1 standard deviation.

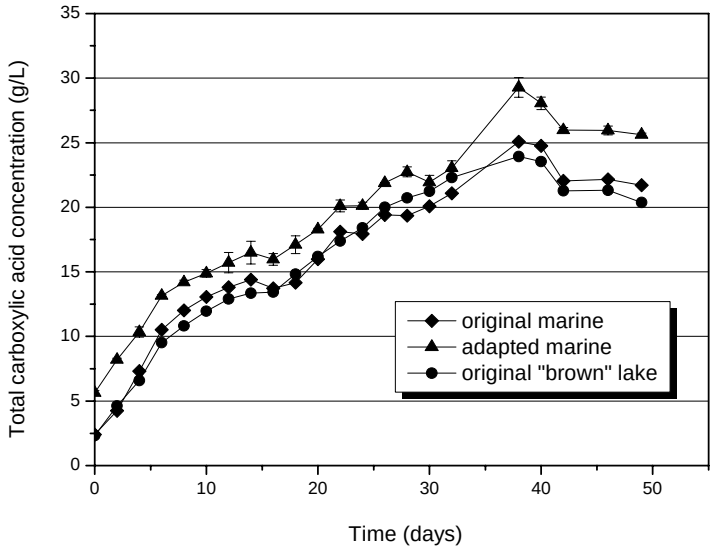


Figure 6-16. Comparison of the total acid concentration of fermentations inoculated from different inoculum sources with 80 g/L of 80% lime treated bagasse/20% chicken manure under thermophilic condition (55°C). Error bar is for duplicate and indicates 1 standard deviation.

b) Effect on acetic acid percentage

Figure 6-17 compares the different salt lake inoculum sources whereas Figure 6-18 compares the different acetic acid percentages for the marine inoculum and the salt lake inoculum sources at 55°C. There was no obvious difference found for those fermentations. All fermentations had similar performance under thermophilic conditions and achieved final acetic acid percentages of nearly 85% in all cases.

c) Summary of the thermophilic fermentation

Table 6-6 summarizes the fermentation results under thermophilic conditions. The fermentation using the "original" mixture of salt lake inocula sources had the "best" fermentation performance among all salt lake inocula sources studied under thermophilic conditions. The marine inoculum sources had similar VS conversion, but higher yield and higher selectivity than the fermentation inoculated with salt lake inocula. The similar conversion of biomass for both marine and salt lake inocula sources at 55°C showed that similar amounts of biomass were consumed by the microorganisms. Because the carboxylic acids are intermediate products for methane, a lower yield of the

Table 6-6. Effect of inoculum sources on thermophilic fermentations.

Inoculum source	Peak acids concentration (g/L)	Peak acids production* (g/L)	Final acids Concentration (g/L)	VS conversion (g/g)	Yield (g acids/g VS)	Selectivity (g acids/g VS)
Original Black lake	21.81 ± 0.16	19.57 ± 0.23	19.35 ± 1.34	0.61 ± 0.01	0.31 ± 0.00	0.51 ± 0.00
Original Brown lake	23.93 ± 2.33	21.61 ± 2.02	20.373 ± 0.976	0.60 ± 0.01	0.34 ± 0.03	0.57 ± 0.05
Original mixture lake	25.73 ± 1.53	23.29 ± 1.41	21.248 ± 1.483	0.64 ± 0.03	0.37 ± 0.02	0.58 ± 0.01
Original marine	25.07	22.67	21.717	0.62	0.36	0.58
Adapted marine	29.29 ± 0.77	23.63 ± 0.93	25.628 ± 0.116	0.60 ± 0.02	0.38 ± 0.02	0.63 ± 0.05

* acid production = measured acid concentration – initial acid concentration
Error bar (±) indicates 1 standard deviation.

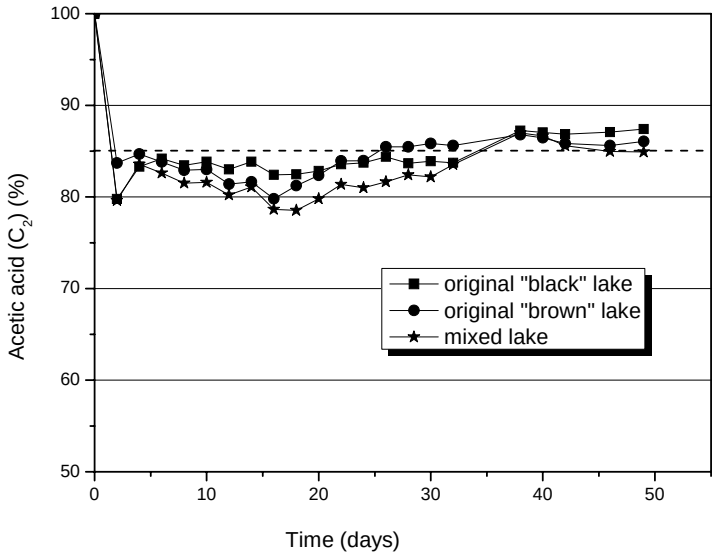


Fig 6-17. Comparison of acetic acid (C2) percentage for fermentations inoculated from different lake inoculum sources with 80 g/L of 80% lime treated bagasse/20% chicken manure under thermophilic condition (55°C).

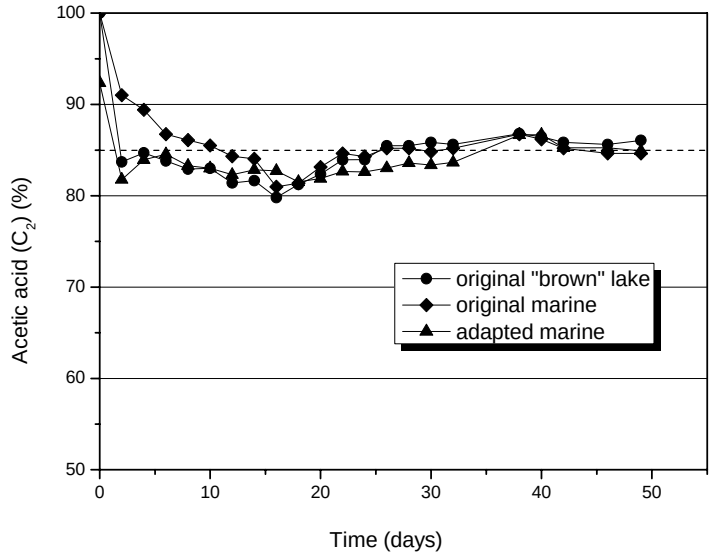


Fig 6-18. Comparison of acetic acid (C2) percentage for fermentations inoculated from marine inoculum sources and salt lake inoculum sources with 80 g/L of 80% lime treated bagasse/20% chicken manure under thermophilic condition (55°C).

total carboxylic acids in the fermentation inoculates from the lake inoculum source hinted that some breakdown reaction of the carboxylic acids may happen due to methane production

At 55°C, the marine inoculum had similar performance to the lake inoculum. The original salt lake inoculum did not show trends similar to the mesophilic fermentations (40°C) which was nearly a 30% increase in total carboxylic acid concentration. The reason for this difference is not yet identified. As shown in Figure 6-19, biomass digestion to methane occurs in three steps: (1) hydrolysis and acidogenesis, (2) acetogenesis and dehydrogenation, and (3) methanogesis. The difference may happen in the carboxylic acids production stage or the methane production stage. Acid-producing microorganisms from different inoculum sources will prefer specific temperatures. Therefore, those microorganisms may have more activity at 40°C than that at 55°C. Secondly, the other possible reason could be the "methanogens," microorganisms that generate methane by metabolizing organic materials, including various hydrocarbons. Methane production in the lake inoculum at 55°C occurred even with the addition of 4.8 mg iodoform/(L·day) as shown in Table 6-4.

Methane production was only detected for salt lake inoculum fermentations at 55°C but not at 40°C. This may be the reason why the original lake system showed better performance at 40°C, but there were no obvious advantages at 55°C. The continuously detected methane production and similar acid concentrations as the marine inoculum could show that the original salt lake inoculum is a potentially better inoculum because the fermentation could be further improved by inhibiting methane production. If methane production could be completely inhibited in the fermentations inoculated with the salt lake inoculum sources, a higher total acid concentration should be expected. The original salt lake inocula sources are promising under thermophilic conditions and still require future improvement.

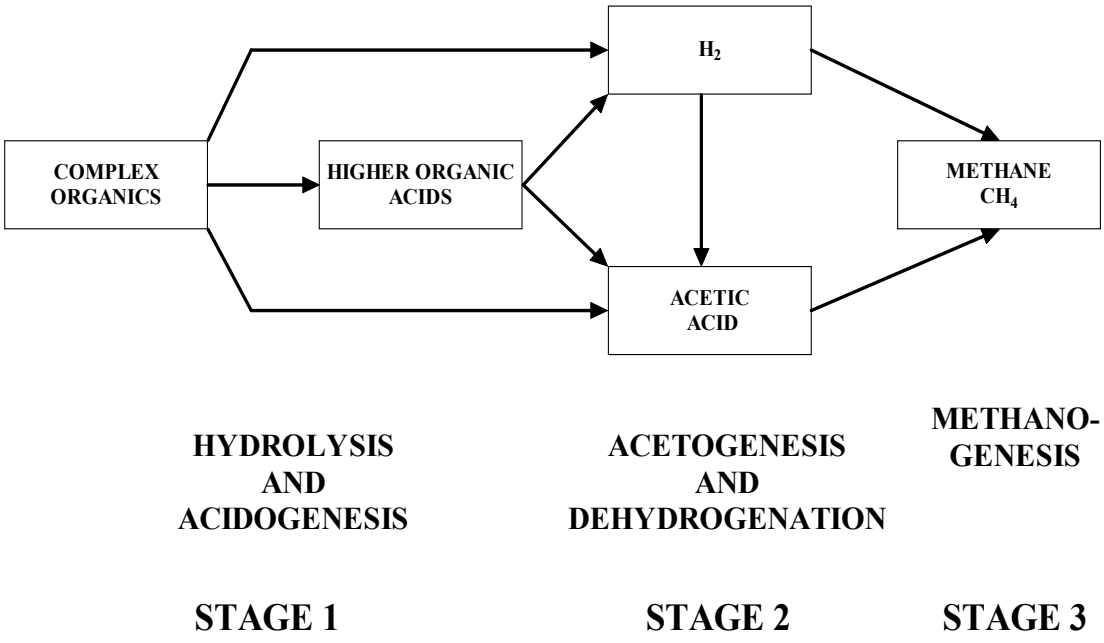


Figure 6-19. The stages of anaerobic fermentations (David P. Chynoweth 1987).

Stricter methane inhibition requirements under thermophilic conditions could be a problem for the salt lake inoculum if we prefer adding the least amount of methane inhibitor as possible. If methane is a preferred product, the original salt lake system could be an "ideal" choice because it can continuously produce methane, even with a high methane inhibitor addition of 4.8 mg/(L·day).

In conclusion, the lake inoculum sources had better performance under the mesophilic conditions (40°C) and similar performance under thermophilic conditions (55°C). This comparable performance of the lake inoculum sources in the anaerobic fermentation compared with the marine inoculum sources showed that the inocula sources from the Great Salt Lake, UT did work in the fermentations buffered by ammonium bicarbonate. Our assumption of the more "robust" microorganisms in higher salt concentrations level environments was valid under mesophilic conditions.

6.3.3 Effect of temperature on fermentation performance

Temperature is vital to the growth of microorganisms. Different microorganisms have their particular optimum temperature where activity is maximal. In this chapter, the microorganism culture from the selected inoculum sources is a mixed culture. The effect of temperature on this mixed culture results from the interaction of the different kinds of microorganisms in the culture and therefore is relatively complex compared to single-strain microorganisms. Different temperatures lead to different product distributions. Some basic understanding of temperature effects on the mixed culture fermentation is the goal of this section. Experimental data from Section 6.3.2 were analyzed again in this section based on the temperature effect.

Effect on total acid concentration

Figure 6-20 shows the influence of temperature on the total acid concentrations. The four subfigures compare four different inoculum sources: the original "black" lake inoculum, the original "brown" lake inoculum, the original "marine" inoculum, and the adapted marine inoculum. Thermophilic fermentations (e.g., 55°C) have higher peak total acid concentrations compared with mesophilic fermentations (e.g., 40°C). For the original "black" lake inoculum source, the peak (i.e., highest) total acid concentration was 17.2 g/L at 40°C compared with 21.8 g/L at 55°C. For the adapted marine inoculum source, the peak total acid concentration for the mesophilic and thermophilic conditions were 18.8 g/L and 29.3 g/L, respectively.

Different inoculum sources showed different responses to temperature. For the original salt lake inoculum sources, mesophilic fermentations exhibited better performance than the thermophilic fermentations in the first 3 weeks, but they showed worse performance than thermophilic fermentations after 3 weeks. For the marine inoculum source, their trends were different from the lake inoculum sources. The measured total acid concentrations were always higher at 55°C than that at 40°C.

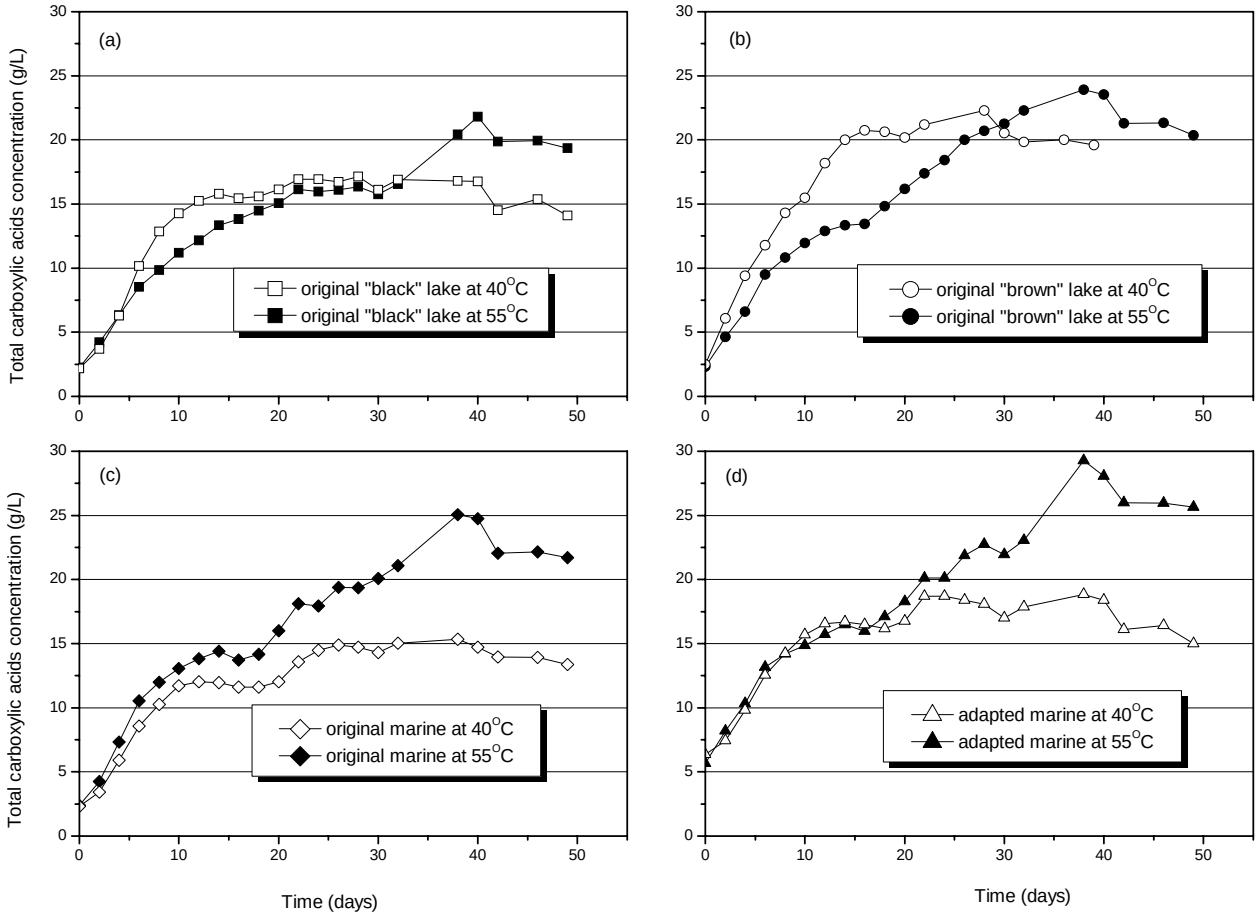


Figure 6-20. Comparison of the total acid concentration for different temperatures with 80 g/L of 80% lime treated bagasse/20% chicken manure. (a) original "black" lake inoculum source, (b) original "brown" lake inoculum source, (c) original marine inoculum source, and (d) adapted marine inoculum source.

If the residence time of the fermentation was less than 3 weeks, the salt lake inoculum produced higher concentration of total carboxylic acids under mesophilic conditions than thermophilic conditions. Furthermore, no methane was detected at 40°C for the lake inoculum sources; therefore, no excess methane inhibitor was required. Lake inocula could be an ideal inoculum source under thermophilic conditions if the residence time is less than 3 weeks.

Effect on acetic acid

Acetic acid (C2) is the major product in the fermentation broth and reached around 90% in some cases. Figure 6-21 shows that the peak acetic acid percentage increased when the temperature increased from 40°C to 55°C for all the selected inoculum sources. In the first 3 weeks, the acetic acid percentages were very similar for different temperatures for most inoculum sources. Only the original marine inoculum showed higher acetic acid selectivity at 55°C than that at 40°C. After the first three weeks, there was some significant increase under the thermophilic conditions for all the selected inoculum sources.

Summary of fermentation performance

Table 6-7 summarizes the final fermentation results based on temperature effects. The thermophilic fermentations inoculated from the marine inoculum sources had a higher VS conversion, higher yield, and higher selectivity than the mesophilic fermentations. For the lake inoculum sources at higher temperature, no significant difference of VS conversion was observed, but the higher temperature did lead to higher yield and selectivity.

In summary, relatively higher VS conversion, higher yield, and higher selectivity were obtained under thermophilic conditions than under mesophilic conditions. The thermophilic fermentation has a more rapid reaction rate, which may reduce the residence time and the reactor size, and therefore decrease the capital cost for the fermentor.

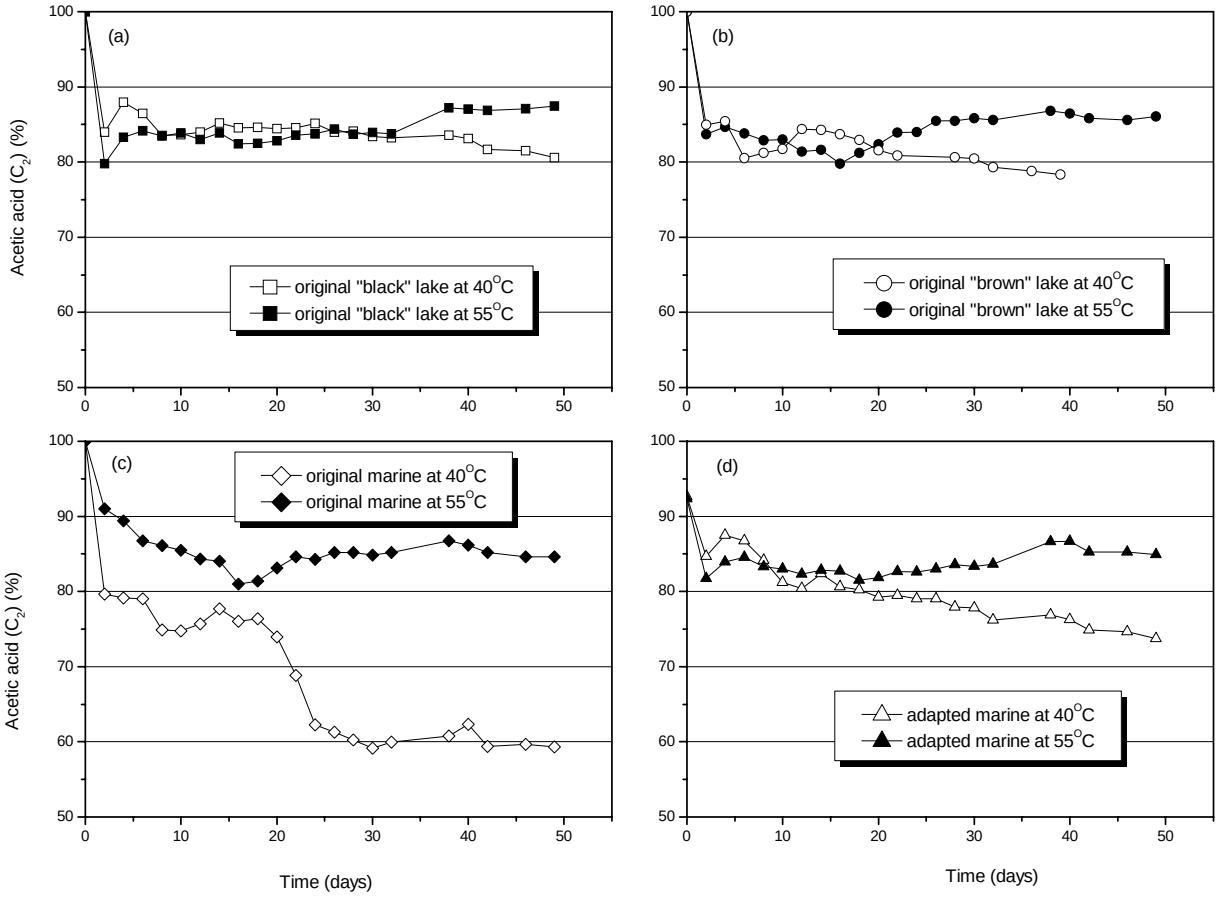


Figure 6-21. Comparison of the acetic acid percentage for different temperatures with 80 g/L of 80% lime treated bagasse/20% chicken manure. (a) original "black" lake inoculum source, (b) original "brown" lake inoculum source, (c) original marine inoculum source, and (d) adapted marine inoculum source.

Table 6-7. Effect of temperature on anaerobic fermentations.

Inoculum source	Fermentation temperature (°C)	Peak acid concentration* (g/L)	peak acid production (g/L)	Final acid concentration (g/L)	VS conversion (g/g)	Yield (g acids/g VS)	Selectivity (g acids/g VS)
Black lake	40	17.23 ± 0.93	15.04 ± 0.92	14.1 ± 3.0	0.60 ± 0.03	0.24 ± 0.02	0.40 ± 0.05
	55	21.81 ± 0.16	19.57 ± 0.23	19.35 ± 1.34	0.61 ± 0.01	0.31 ± 0.00	0.51 ± 0.00
Brown lake	40	22.30	19.81	19.60	0.60	0.27	0.44
	55	23.93 ± 2.33	21.61 ± 2.02	20.37 ± 0.98	0.60 ± 0.01	0.34 ± 0.03	0.57 ± 0.05
Original marine	40	15.33	13.03	13.39	0.57	0.21	0.37
	55	25.07	22.67	21.72	0.62	0.36	0.58
Adapted marine	40	18.82	12.46	14.99	0.58	0.20	0.34
	55	29.29 ± 0.77	23.63 ± 0.93	25.63 ± 0.12	0.60 ± 0.02	0.38 ± 0.02	0.63 ± 0.05

* acid production = measured acid concentration – initial acid concentration

Error bar (±) indicates 1 standard deviation.

6.4 Conclusions

The following conclusions can be made based on the present study in this chapter:

1) The lake inocula from the Great Salt Lake, UT did work in the anaerobic fermentation under both thermophilic conditions (55°C) and mesophilic conditions (40°C). Under mesophilic conditions, it had a comparable or better performance than the marine inocula. This confirmed the assumptions that the "robust" microorganisms acclimated to the high salt concentration in the Great Salt Lake may be well suited to the anaerobic fermentations of the MixAlco process.

2) Under mesophilic conditions (40°C), the original "brown" inoculum from the Great Salt Lake exceeded the marine inocula, including the original source and adapted source. The concentration of total carboxylic acids increased around 30%; however, there was no significant difference between the marine sources and the lake sources under thermophilic conditions (55°C). This could be explained by the detected methane production in the thermophilic fermentations but no methane detected in the mesophilic fermentations.

3) Thermophilic fermentations (55°C) obtained a higher reaction rate and higher acetic acid percentage compared with mesophilic fermentations (40°C). For the adapted marine inocula, there is no obvious difference in the first 3 weeks of the thermophilic fermentations compared with the mesophilic fermentations. After 3 weeks, some significant difference occurred. On Day 46, the thermophilic fermentation obtained a higher total carboxylic acids concentration of 25.9 g/L compared with 16.4 g/L under mesophilic condition (40°C) for the initial 80 g/L 80% lime-treated bagasse/20% chicken manure. A higher acetic acid percentage 85% was achieved at 55°C compared with 75% at 40°C.

CHAPTER VII
INTRODUCTION AND PRINCIPLES OF COUNTERCURRENT FERMENTATIONS AND CPDM MODEL

The objectives of this chapter follow:

a) To introduce the basic principles of countercurrent fermentations in the MixAlco process.

b) To describe the Continuum Particle Distribution Model (CPDM)

c) To show the required batch experimental procedure used to obtain model parameters for CPDM prediction.

d) To describe the method used to predict the conversion and product concentration "map."

e) To compare two different computer programs (*Mathematica* program and *Matlab* program) for CPDM method.

7.1 Countercurrent fermentations

Anaerobic fermentation is the core of the MixAlco process. During a typical fermentation, the treated biomass is inoculated with a mixed culture of anaerobic microorganisms. The biomass feedstock is digested by anaerobic microorganisms that produce carboxylic acids (e.g., acetic acids, propionate acids, and butyric acids). End product inhibition is always an issue in batch fermentations, whereas it can be mitigated via countercurrent fermentations (Holtzapple et al. 1996; Holtzapple et al. 1997).

High conversions and high product concentrations in the fermentation are possible using countercurrent operation (Ross and Holtzapple 2001). The laboratory countercurrent fermentations deploy rotary fermentors (1-L centrifuge bottles). Figure 7-1 shows the pilot-scale fermentors for countercurrent operation. Countercurrent fermentations (Figure 7-2) allow the least reactive biomass to contact the lowest carboxylic acid concentration, which in batch fermentations cannot be digested because of carboxylic acid accumulation. As the solids are transferred from one fermentor to the next upstream fermentor (i.e., from F1 to F2, F2 to F3, and F3 to F4), the biomass becomes less reactive and the carboxylate salt concentration becomes lower. Figure 7-3 shows the steady-state product distribution in a typical laboratory countercurrent fermentation. The total carboxylic acid concentration at steady state in F1, F2, F3, and F4 is 28.9, 20.3, 17.2, and 5.5 g/L, respectively. Therefore, fresh biomass contacts the highest acid concentration (28.9 g/L) in Fermentor F1 and fresh liquid can contact the lowest acid concentration (5.5 g/L) in Fermentor F4. This countercurrent flow arrangement reduces the inhibitory effect from the accumulation of product carboxylate salts by adding fresh liquid to the most digested biomass in F4.

In conclusion, countercurrent fermentation greatly reduces possible end product concentration inhibition; therefore, it is preferred for long-term continuous operation in the MixAlco process.

Figure 7-1. Photograph of countercurrent fermentation reactors in pilot plant (College Station, TX).

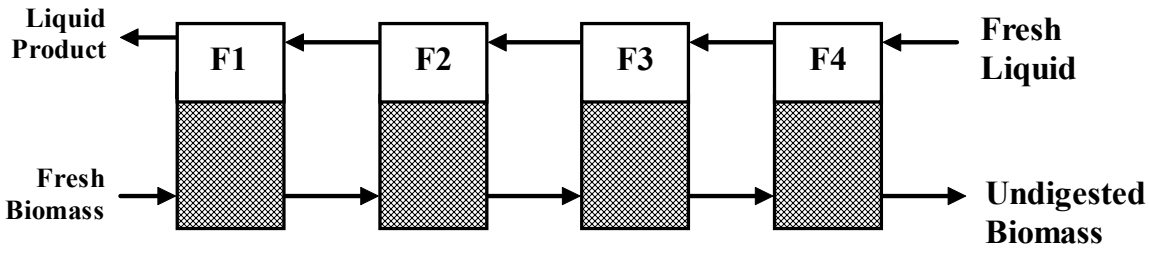


Figure 7-2. Schematic flowsheet for a typical four-stage countercurrent fermentation.

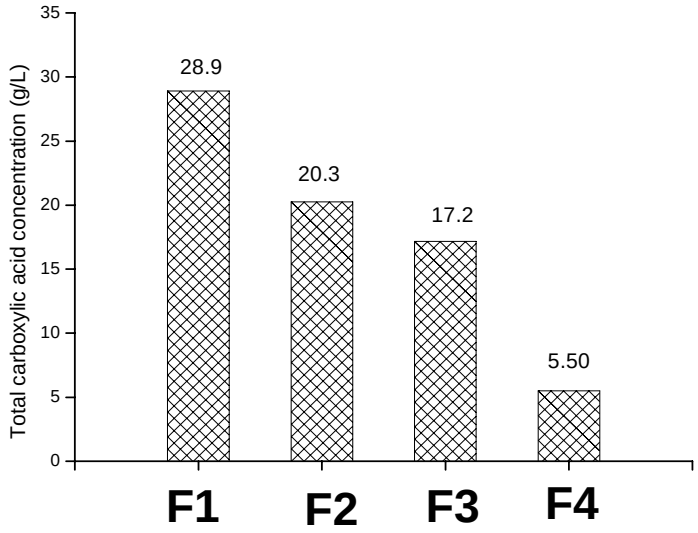


Figure 7-3. Steady-state product concentrations in a typical four-stage countercurrent fermentation of 80 wt% hot-lime-water-treated bagasse/20% chicken manure at LRT of 28.1 days and VSLR of 4.5 g/(L·d). Calcium carbonate was used as buffer.

7.2 Principles of CPDM method

Countercurrent fermentations in the laboratory are time-consuming. It may take several weeks to months to achieve the final steady state. Furthermore, long residence times are associated with fermentation systems. Thus, the optimization of fermentation for a single feedstock could take years and would require thousands of man-hours. The Continuum Particle Distribution Model (CPDM) method developed by Loescher (1996) has been used to predict the product concentration and biomass conversions for countercurrent fermentations (Agbogbo 2005; Aiello Mazzarri 2002; Thanakoses 2002).

The CPDM method has initially been used to quantify the kinetics of a reaction occurring at the interface between solid and fluid phases. Some examples are microbial coal desulfurization, coal combustion, and enzymatic hydrolysis. The CPDM method utilizes data collected from batch experiments to predict product concentrations and conversions for various solid loadings and residence times. The CPDM method has been found to predict values within 10–20% of the experimental results for different biomass fermentations (Agbogbo 2005; Aiello Mazzarri 2002; Thanakoses 2002).

The concept of *continuum particle* is used in CPDM method 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 the following two 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 definition and describes a *continuum particle* as a collection of particles that has 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 7-1.

$$S_0 = \int_0^1 \hat{n}(x)dx \tag{7-1}$$

Equation 7-2 relates the total reaction rate (r) with the specific rate ($\hat{r}$) as a function of particle conversion and product concentrations A. The specific rate $\hat{r}(x, A)$ contains information about the reacting system and products and $\hat{n}(x)$ contains information about substrate concentrations and conversions.

$$r = \int_0^1 \hat{r}(x, A)\hat{n}(x)dx \tag{7-2}$$

For a batch reaction, all particles have the same conversion. Therefore, $\hat{n}(x) = 0$ everywhere except at x'.

$$n_0 = \int_0^1 \hat{n}(x)dx = \lim_{\varepsilon \to 0} \int_{x'-\varepsilon}^{x'+\varepsilon} \hat{n}(x)dx \tag{7-3}$$

The Dirac delta function can be used to represent the distribution function as in Equation 7-4.

$$\hat{n}(x) = S_0\delta(x - x') \tag{7-4}$$

Substituting this particle distribution into Equation 7-2 gives Equation 7-5.

$$r = \int_0^1 \hat{r}(x, A)\hat{n}(x)dx = \int_0^1 \hat{r}(x, A)S_0\delta(x - x')dx = \hat{r}(x', A)S_0 \tag{7-5}$$

In conclusion, the CPDM model relates the reaction rate with some constant model parameters obtained from batch fermentations. The batch fermentation procedure for CPDM model parameters is detailed in Section 7.3. With those model parameters, the CPDM method could determine the optimum volatile solid loading rate (VSLR) and liquid residence time (LRT) in a short time (i.e., batch fermentation time of 15 □ 30 days) (Aiello Mazzarri 2002; Thanakoses 2002).

7.3 Batch experiments to obtain model parameters for CPDM method

In general, it takes 15 to 20 days to obtain the batch fermentation data needed for the CPDM model. Batch experiments consist of five fermentors run simultaneously with different initial substrate concentrations. The substrate concentrations used were 40, 70, 100, and 100+ g substrate/L liquid. The 100 and 100+ fermentors had the same initial substrate concentration, but the 100+ fermentor contained a medium with a mixture of carboxylate salts in a concentration of approximately 20 g of carboxylic acids/L liquid. Table 7-1 lists the components and distribution of mixed carboxylate salts used in batch fermentations. Two formulas of carboxylate salts were used: 100+ (a) and 100+ (b). 100+ (a) formula in Table 7-1 followed the common 70% acetate content in calcium carbonate buffered fermentations, whereas 100+ (b) formula considered the common 85% acetate content in ammonium bicarbonate fermentation. Calcium butyrate was used to replace ammonium butyrate in ammonium bicarbonate batch fermentations, because there is no ammonium butyrate available in the market.

Table 7-1. The carboxylate salts used in 100+ fermentor.

Formula	Weight ratio of acetate salts	Weight ratio of propionate salts	Weight ratio of butyrate salts
100+ (a)			
for NH_4HCO_3 fermentation	70% NH_4^+ salt	20% NH_4^+ salt	10% Ca^{2+} salt
for $CaCO_3$ fermentation	70% Ca^{2+} salt	20% Ca^{2+} salt	10% Ca^{2+} salt
100+ (b)			
for NH_4HCO_3 fermentation	85% NH_4^+ salt	5% NH_4^+ salt	10% Ca^{2+} salt
for $CaCO_3$ fermentation	85% Ca^{2+} salt	5% Ca^{2+} salt	10% Ca^{2+} salt

The inoculum for batch fermentors was taken from countercurrent fermentations operating with the same substrate, so that the microorganisms were already adapted to this type of substrate. The initial carboxylic acid concentration in batch fermentors resulted from the acids contained in the initial inoculum. Both dry nutrient mixture and methane inhibitor were initially added as the same pattern with the countercurrent operation. The pH, gas production, and gas composition were monitored during batch experiments. Iodoform was added each other day to inhibit methane production. Daily samples of the liquid were taken from each fermentor, and the amount of carboxylic acid produced was measured by gas chromatography (Chapter II).

The carboxylic acid concentrations detected by gas chromatography can be converted into acetic acid equivalents (Aceq). Aceq represents the amount of acetic acid that could have been produced in the fermentation if all the carboxylic acids produced 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 7-6 through 7-10 are used to calculate the Aceq concentration.

Propionic acid: $7\ HOAc \longrightarrow 4\ HOPr + 2\ CO_2 + 2\ H_2O$ (7-6)

Butyric acid: $5\ HOAc \longrightarrow 2\ HOBu + 2\ CO_2 + 2\ H_2O$ (7-7)

Valeric acid: $13\ HOAc \longrightarrow 4\ HOVa + 7\ CO_2 + 6\ H_2O$ (7-8)

Caproic acid: $4\ HOAc \longrightarrow HOCa + 2\ CO_2 + 2\ H_2O$ (7-9)

Heptanoic acid $19\ HOAc \longrightarrow 4\ HOHe + 10\ CO_2 + 10\ H_2O$ (7-10)

In batch fermentations for CPDM parameters, the liquid sample was required to be analyzed twice to obtain the average value. After the liquid samples were analyzed, the average carboxylic acid concentration was converted into Aceq by using Equations 7-11 and 7-12. A *Perl* script code (Appendix M) was used to automatically convert the duplicate total carboxylic acid concentration in the GC EXCEL file to average Aceq.

$$\alpha\ (\text{mol/L}) = 1.0\ (\text{acetic}) + 1.75\ (\text{propionic}) + 2.5\ (\text{butyric}) + 3.25\ (\text{valeric}) + 4.0\ (\text{caproic}) + 4.75\ (\text{heptanoic}) \quad (7\text{-}11)$$

$$\text{Aceq}\,(\text{g/L}) = 60.05 \times [\alpha\,(\text{mol/L})] \quad (7\text{-}12)$$

The concentrations of acetic acid equivalents Aceq(t) in each batch experiment are fit to Equation 7-13, where a, b, and c are constants fit by least squares regression, and t is the fermentation time in days. Initial value for the parameters a, b, and c can be guessed in this calculation.

$$\text{Aceq} = a + \frac{bt}{1 + ct} \quad (7\text{-}13)$$

The residuals are defined as the difference between the experimental and calculated Aceq values. The residuals are minimized and the parameter values of a, b, and c are obtained.

$$\text{Residuals} = \sum_{\text{data}} (\text{Aceq}_{\text{exp}} - \text{Aceq}_{\text{calculated}})^2 \quad (7\text{-}14)$$

The reaction rate for the fermentation is then determined by the equation

$$r = \text{rate} = \frac{d\,(\text{Aceq})}{dt} = \frac{b}{(1 + ct)^2} \quad (7\text{-}15)$$

The specific reaction rate ($\hat{r}$, the reaction rate per particle) is calculated by the reaction rate in Equation 7-15 divided by the initial substrate concentration (S_o) in the respective batch fermentor.

$$\hat{r} = \frac{r}{S_o} \tag{7-16}$$

where S_o, the initial amount of substrate (g VS/L), is defined as $S_o = m_o/V$. In batch fermentations, m_o is the initial substrate mass (g VS), V is the liquid volume in the batch fermentor (L). However, in a typical four-stage countercurrent fermentation, m_o is the mass of fresh biomass added to Fermentor 1, and V is defined as the fresh liquid volume added to Fermentor 4.

The biomass conversion (x) is calculated for each batch fermentor, using Equation 7-17.

$$x(t) = \frac{\text{Aceq}(t) - \text{Aceq}(t = 0)}{S_o \sigma} \tag{7-17}$$

where σ is the selectivity (g Aceq produced/g VS digested). In the CPDM method, the selectivity σ is assumed as constant and calculated from the selectivity s by equation 7-18. The average value of selectivity s (g total acid produced/g VS digested) is determined from the countercurrent experiments.

$$\sigma = \frac{s}{\phi} \tag{7-18}$$

Equation 7-19 is the governing equation deployed in the CPDM method. It relates the specific reaction rate $\hat{r}(x, Aceq)$ with Aceq concentration ($Aceq$) and conversion (x).

$$\hat{r}_{pred} = \frac{e(1-x)^{f}}{1+g(\phi \cdot \mathrm{Aceq})^{h}} \tag{7-19}$$

where x = fraction conversion of volatile solids

$e, f, g,$ and h = empirical constants

ϕ = the ratio of total grams of carboxylic acid to total grams of acetic acid equivalents

Equation 7-19 is an empirical equation. South and Lynd (1994) described the (1–x) term in equation 7-19 as the *conversion penalty function*. This term (1–x) shows that, as the substrate is converted, the reaction rate decreases. The denominator term in equation 7-19 describes the inhibitory effect of end product concentration on the microorganisms, which decreases the reaction rate. Ross (1998) introduced parameter ϕ to avoid the inhibitory effects of higher acids that would overestimate the specific rate.

The values of Aceq, the specific reaction rate $\hat{r}$, and conversion x are obtained from the experimental data of batch fermentations. That is to say, Aceq is obtained from Equation 7-12, the specific reaction rate from Equation 7-16, and the conversion from Equation 7-17, respectively. Parameter values of e, f, g, and h in Equation 7-19, are fit by non-linear regression (*SYSSTAT SIGMAPLOT 10.0*) to minimize the experimental value and the predicted value of the specific reaction rate $\hat{r}(t)$.

In conclusion, the batch fermentations are set up to obtain the parameter values of e, f, g, and h in the governing equation (Equation 7-19). The other required system-specific parameters for CPDM method are selectivity (σ), holdup (ratio of liquid to solids in wet solids), and moisture (ratio of liquid to solids in feed solids). Based on

these parameters, the *Mathematica* or *Matlab* program for CPDM method (Appendices H and I) can predict the Aceq concentration and conversion (x) for countercurrent fermentations at various volatile solid loading rates (VSLR) and liquid residence times (LRT).

7.4 Conversion and product concentration "map"

As mentioned in Section 7.3, the CPDM model can predict the final product conversion and carboxylic acid concentration based on the preset LRT and VSLR. With the results obtained from every computer run, a "map" was drawn to show the dependence of the substrate conversion and product concentration for various VSLR and LRT. This "map" provides a visual relationship between conversion and product concentrations and was obtained through a self-coded *Matlab* program (Appendix J).

This *Matlab* program can be used standalone, if the conversion and product concentration are provided. It also can be combined in the CPDM *Matlab* program to automatically draw the "map" as a standard output.

7.5 Comparison of CPDM prediction using *Matlab* program and *Mathematica* program

The *Mathematica* program (Appendix H) and *Matlab* program (Appendix I) for CPDM prediction were compared to examine the CPDM prediction performance. Matlab® version R2006b (http://www.mathworks.com) was used for *Matlab* program, whereas Mathematica® version 5.1 (http://www.wolfram.com) was used for *Mathematica* program. Both programs were running in a personal computer with Windows XP Professional version, 2.8-GHz Intel Core Dual CPU, and 2 GB DDR-533 memory.

Table 7-2. Parameter constant values used in CPDM prediction comparison.

Parameter constant	Value
VSLR (g/(L liquid·day))	7.5
LRT (day)	14.0
Holdup (g liquid/g VS in wet cake)	1.87
Moisture (g liquid/g VS in feed)	1.1
Selectivity (g Aceq/g VS digested)	0.6
F1–F4 solid concentration (g VS/L)	169, 214, 214, and 214
F1–F4 liquid volume (L)	0.48, 0.24, 0.24, and 0.24
ϕ (g total acid/g Aceq)	0.8
e (g Aceq/(g VS·day))	0.141
f (dimensionless)	2.01
g (L/g total acid)$^{1/h}$	5.17
h (dimensionless)	0.273

Table 7-2 lists the system-specific model variables required in the prediction comparison of both programs, whereas Table 7-3 summarizes the Aceq concentrations and conversions for countercurrent fermentations calculated by *Mathematica* program and *Matlab* program. Table 7-3 shows that the product concentration and conversion calculated by *Mathematica* program agree well with *Matlab* program (absolute error < 0.2%).

Table 7-3. Comparison of the calculated carboxylic acid concentrations and conversions by *Mathematica* program and *Matlab* program.

	F1 concentration (g/L)	F2 concentration (g/L)	F3 concentration (g/L)	F4 concentration (g/L)	Average** (%)
Mathematica prediction	27.5847	21.3444	14.4605	7.4239	
Matlab prediction	27.5822	21.2451	14.4154	7.4427	
Difference* (%)	0.01	0.47	0.31	-0.25	
	F1 conversion	F2 conversion	F3 conversion	F4 conversion	
Mathematica prediction	0.1170	0.1898	0.2631	0.3406	
Matlab prediction	0.1170	0.1899	0.2629	0.3401	
Difference* (%)	-0.06	-0.02	0.07	0.16	0.17

* Difference (%) = ((*Mathematica* prediction – *Matlab* prediction)/*Matlab* prediction) × 100

** Average difference is based on absolute value.

Part of the output from *Mathematica* program is shown as follows:

```
{191.382,264.148,290.41,324.528}
                    acid 1 = 26.5006   taulnew 1 = 5.6349 robs = 1.76804
 nhatzero= 100  nhattot= 275.244 nnot[[i]]= 264.148
 nhatzero= 97.8996  nhattot= 275.011 nnot[[i]]= 264.148
 nhatzero= 95.8433  nhattot= 274.783 nnot[[i]]= 264.148
 nhatzero= 93.8303  nhattot= 274.559 nnot[[i]]= 264.148
 nhatzero= 91.8595  nhattot= 274.341 nnot[[i]]= 264.148
 nhatzero= 89.9301  nhattot= 274.126 nnot[[i]]= 264.148
 nhatzero= 88.0412  nhattot= 273.917 nnot[[i]]= 264.148
 nhatzero= 86.192  nhattot= 273.712 nnot[[i]]= 264.148
 nhatzero= 84.3816  nhattot= 273.511 nnot[[i]]= 264.148
…….
{191.39,264.147,290.416,324.582}
                    acid 1 = 27.5847   taulnew 1 = 5.5716 robs = 1.75448
 nhatzero= 2.96293  nhattot= 264.471 nnot[[i]]= 264.147
                    acid 2 = 21.3444   taulnew 2 = 2.63599 robs = 2.18538
 nhatzero= -0.271278  nhattot= 290.736 nnot[[i]]= 290.416
                    acid 3 = 14.4605   taulnew 3 = 2.6785 robs = 2.19815
 nhatzero= -0.31625  nhattot= 324.885 nnot[[i]]= 324.582
                    acid 4 = 7.42389   taulnew 4 = 2.7185 robs = 2.32673
 conversion in each stage (from nhat) {0.116965,0.189811,0.263083,0.34064}
 0.0857745
 0.0907362
 0.0895094
 0.0882764
 0.0869725
 Selectivity =  {709.194,796.702,809.608,806.971}
 Creation =  {0.74656,0.522329,0.533891,0.563281}
 destruction =  {0.00105269,0.000655614,0.000659444,0.000698019}
 selectivity =  0.771769
 k =  3.5    l =  1
 loading =  7.5
 tauloverall   14
 taus   33.6514
 acid levels   {27.5847,21.3444,14.4605,7.42389}
```

Part of the output from *Matlab* program is shown as follows:

```
Program starts at:  20-Mar-2005 06:41:18
Calculation is in progress…….
   nnot=     187.77778      267.50000      305.71429      356.66667
   acid(1)= 26.40310  taulnew(1)= 5.60222   robs = 1.74255
  nhatzero=  100.00000 ;   nhattot=  277.83163 ;   nnot(2)=  267.50000
  nhatzero=  92.76786 ;   nhattot=  277.09584 ;   nnot(2)=  267.50000
  nhatzero=  86.05077 ;   nhattot=  274.45823 ;   nnot(2)=  267.50000
  nhatzero=  81.18001 ;   nhattot=  275.40627 ;   nnot(2)=  267.50000
```

```
  nhatzero=  75.64562 ;   nhattot=  275.34747 ;   nnot(2)=  267.50000
  nhatzero=  70.15239 ;   nhattot=  274.82787 ;   nnot(2)=  267.50000
  nhatzero=  65.02289 ;   nhattot=  274.47541 ;   nnot(2)=  267.50000
  nhatzero=  60.14010 ;   nhattot=  273.92509 ;   nnot(2)=  267.50000
  nhatzero=  55.64253 ;   nhattot=  273.40833 ;   nnot(2)=  267.50000
  nhatzero=  51.50670 ;   nhattot=  272.90063 ;   nnot(2)=  267.50000
……
nhatzero=  3.23011 ;   nhattot=  324.12581 ;   nnot(4)=  324.21383
  taulnew(4)=2.73986   taul(4)=2.73974
   acid(4)= 7.44271  taulnew(4)= 2.73986   robs = 2.31583
Conversion in each stage (from nhat):    0.11704      0.18985      0.26289      0.34009
Congratulation! The simulation process is successfully finished!
Elapsed time is 232.515000 seconds.
   L(1)= 0.085719
   L(2)= 0.090966
   L(3)= 0.089283
   L(4)= 0.0876
   L(5)= 0.085917
   SELECTIVITY =709.56110      803.30870      807.50123      805.53989
   Creation = 0.74744        0.52637        0.53084        0.55965
   destruction =0.00105        0.00066        0.00066        0.00069
   selectivity = 0.77245
   tauloverall= 14.00000
   taus  = 33.64092
   acid levels   = 27.58220     21.24506     14.41538      7.44271
```

In conclusion, the *Mathematica* program and *Matlab* program achieved similar product concentration and conversion (absolute error < 0.17%). It depends on personal preference to select the *Mathematica* program or the *Matlab* program. The *Matlab* program (232.5 s) is more time-consuming than the *Mathematica* program (23.1 s), but the *Matlab* program could automatically draw the conversion and production concentration "map" based on the preset LRT and VSLR. In addition, modification of the *Mathematica* program to the *Matlab* program is helpful to examine the understanding of application CPDM methods in countercurrent fermentations. Based on this understanding, further application of CPDM methods could be extended to other fermentation configurations (e.g., liquid-transfer-only fermentations).

CHAPTER VIII
COMPARISON OF AMMONIUM BICARBONATE AND CALCIUM CARBONATE IN COUNTERCURRENT FERMENTATIONS

The objectives of this chapter follow:

a) To examine the long-term effects of ammonium bicarbonate and calcium carbonate on hot-lime-water-treated bagasse fermentations inoculated from marine inocula.

b) To apply the Continuum Particle Distribution Model (CPDM) method to compare the experimental with predicted acid concentration and conversion based on the experimental operation conditions.

c) To predict the "best" performance of industrial fermentor using the CPDM "map."

This chapter is a continued investigation of the experiments described in Chapter III. This chapter focuses on the effects of ammonium bicarbonate and calcium carbonate on long-term continuous fermentations under thermophilic conditions. In this study, 80 wt% of hot-lime-water-treated sugarcane bagasse and 20 wt% of chicken manure were used as substrates in the rotary fermentors. Hot-lime-water treatment (i.e., lime treatment at 100°C with a treatment time of 2 hours) was used in this chapter, whereas air-lime treatment was deployed in Chapter IX. All fermentation trains in this chapter were inoculated from marine (sediments from different locations in Galveston Island, TX). All fermentations were operated at 55°C. Both experimental results and CPDM prediction of carboxylic acid concentration in countercurrent fermentations at various volatile solid loading rates (VSLR) and liquid residence time (LRT) are presented in this chapter.

8.1 Materials and methods

Four-stage countercurrent fermentations were used. Four fermentations were started as batch fermentations with 80 wt% of hot-lime-water-treated sugarcane bagasse and 20 wt% of chicken manure, dry nutrient mixture, and deoxygenated water. Ammonium bicarbonate was the only chemical added to adjust the pH to about 7.0 in ammonium bicarbonate buffered fermentations, whereas calcium carbonate was the buffer used to control pH in calcium carbonate buffered fermentations. Urea (0.1 g) was added as a supplemental nutrient source, if the pH in calcium carbonate buffered fermentations fell below 6.0.

The single-centrifuge procedure, where liquids are transferred in a single step, was used in all fermentation trains. Liquids and solids were transferred at 2-day intervals. After the steady state was achieved (±5 g/L total acid concentration), fermentation data

were collected for at least 10 transfers to determine acid productivity, carboxylic acid concentration, yield, selectivity, conversion, biotic carbon dioxide, and methane production. The total liquid in the fermentation train is the sum of the residual liquid in the wet solid cake and the centrifuged liquid on top of the wet cake. It was determined by first centrifuging each fermentor in a train and separating the solid from the liquid. The residual liquid in the solid cake and the centrifuged liquid were determined also.

8.2 Hot-lime-water-treated bagasse fermentation buffered by calcium carbonate

A series of four countercurrent fermentations (Trains CA, CC, CE, and CF) were performed using calcium carbonate as a buffer. All of the fermentation trains used the same fresh liquid addition (100 mL).

8.2.1 Train CA

Four batch fermentations were initiated by adding hot-lime-water-treated bagasse (32 g), chicken manure (8 g), calcium carbonate (3 g), nutrient mixture (0.2 g), urea (0.1 g), marine inocula (40 mL), anaerobic water (360 mL), and 120 μL iodoform solution (20 g/L of iodoform dissolved in ethanol). The marine inocula were taken from previous batch fermentation of hot-lime-water-treated bagasse and chicken manure using calcium carbonate buffer. On each transfer with Train CA, hot-lime-water-treated bagasse (6.4 g), chicken manure (1.6 g), nutrients (0.2 g), calcium carbonate (2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g), calcium carbonate (2 g), and iodoform (60 μL) were added to F2, F3, and F4. The transfer of solids and liquids was performed as shown in Chapter VII. Fresh anaerobic water (100 mL) was added to F4 on each transfer. Urea (0.1 g) was added as a nitrogen source if the pH in the fermentation broth was below 6.0. The total acid concentration profile and acetate content profile are illustrated in Figures 8-1 and 8-2.

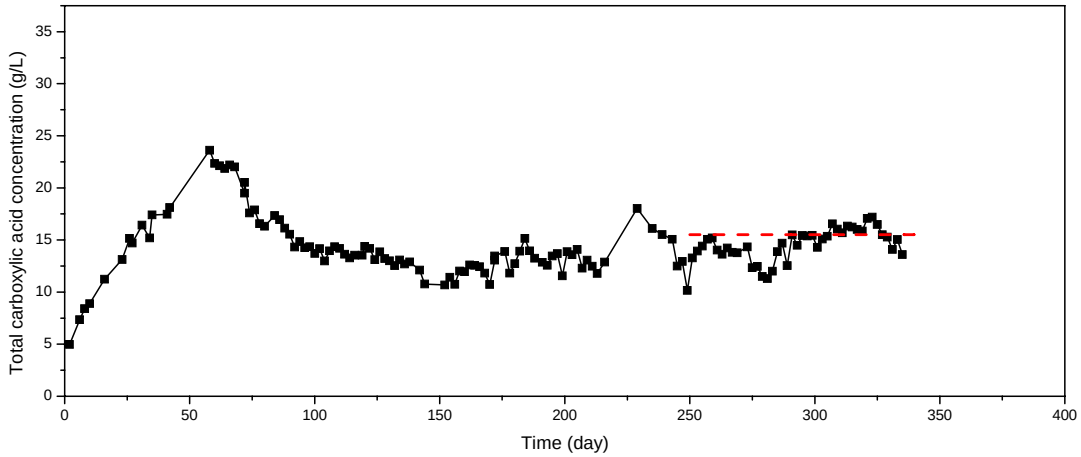


Figure 8-1. Total acid concentration for hot-lime-water-treated bagasse Fermentation Train CA (calcium carbonate, fresh solid 8 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (15.51 g/L).

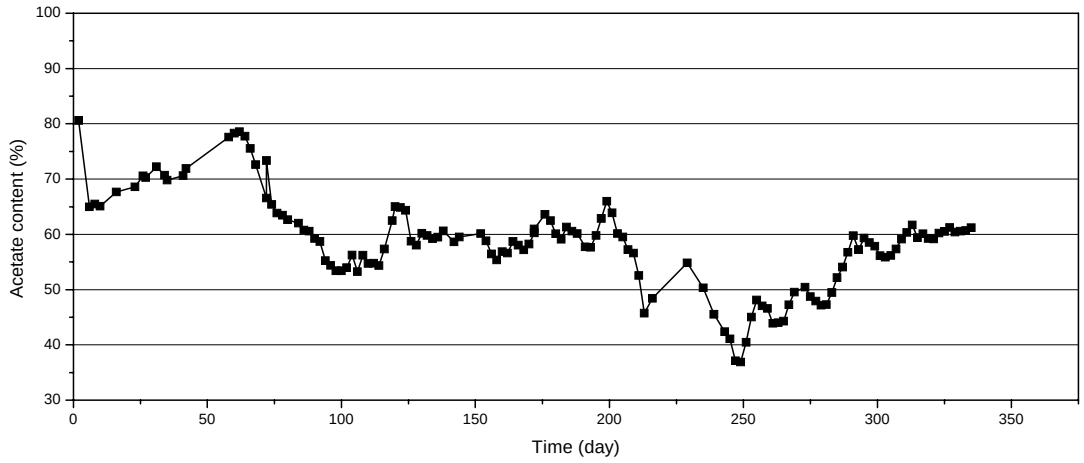


Figure 8-2. Acetate content for hot-lime-water-treated bagasse Fermentation train CA (calcium carbonate, fresh solid 8 g, fresh liquid 100 mL, and constant cake weight 300 g).

8.2.2 Train CC

Four batch fermentations were initiated by adding hot-lime-water-treated bagasse (32 g), chicken manure (8 g), calcium carbonate (3 g), nutrient mixture (0.2 g), urea (0.1 g), 40 mL of marine inocula, anaerobic water (360 mL), and 120 μL iodoform solution (20 g/L of iodoform dissolved in ethanol). The marine inocula were taken from previous batch fermentation of hot-lime-water-treated bagasse and chicken manure using calcium carbonate buffer. On each transfer with Train CA, hot-lime-water-treated bagasse (9.6 g), chicken manure (2.4 g), nutrients (0.2 g), calcium carbonate (2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g), calcium carbonate (2 g), and iodoform (60 μL) were added to F2, F3, and F4. The transfer of solids and liquids was performed as shown in Chapter VII. Fresh anaerobic water (100 mL) was added to F4 on each transfer. Urea (0.1 g) was added as a nitrogen source if pH was below 6.0. The total acid concentration profile and acetate content profile are shown in Figures 8-3 and 8-4.

8.2.3 Train CE

Train CE was started after Train CC was harvested. Four batch fermentations were initiated by even distribution of the harvested solids and liquids from Train CC. Each batch fermentations was started by adding solid cake (80 g) from Train CC, residual liquid (108 mL) from Train CC, hot-lime-water-treated bagasse (32 g), chicken manure (8 g), calcium carbonate (3 g), nutrient mixture (0.2 g), urea (0.1 g), 150 mL of anaerobic water, and 120 μL iodoform solution (20 g/L of iodoform dissolved in ethanol). The residual liquid and residual solids from train CC provided the initial microorganisms to Train CE. On each transfer with Train CE, hot-lime-water-treated bagasse (12.8 g), chicken manure (3.2 g), nutrients (0.2 g), calcium carbonate (2 g), and iodoform (120 μL) were added to F1. nutrients (0.2 g), calcium carbonate (2 g), and iodoform (60 μL) were added to F2, F3, and F4. Fresh anaerobic water (100 mL) was added to F4 on each transfer. Urea (0.1 g) was added as a nitrogen source if the pH in the fermentation broth was below 6.0. The total acid concentration profile and acetate content profile are illustrated in Figures 8-5 and 8-6.

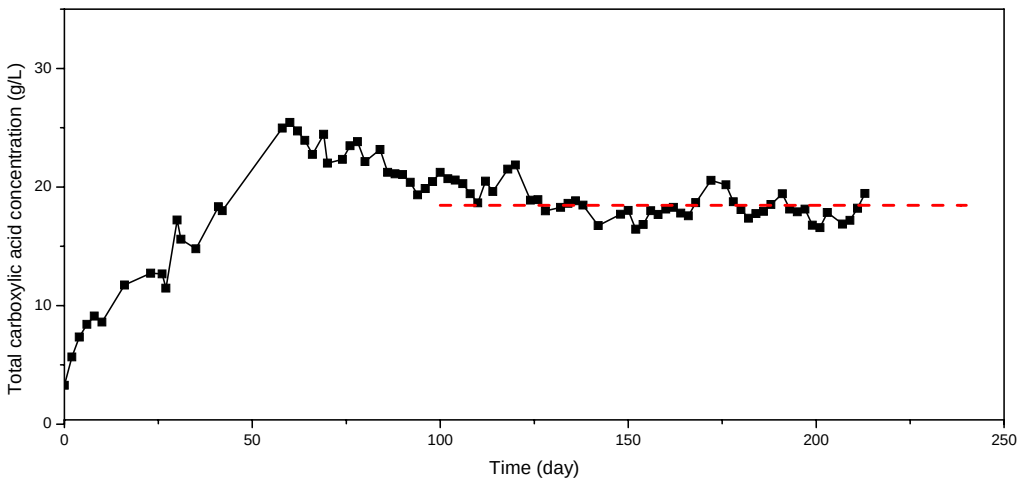


Figure 8-3. Total acid concentration for hot-lime-water-treated bagasse Fermentation Train CC (calcium carbonate, fresh solid 12 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (20.46 g/L).

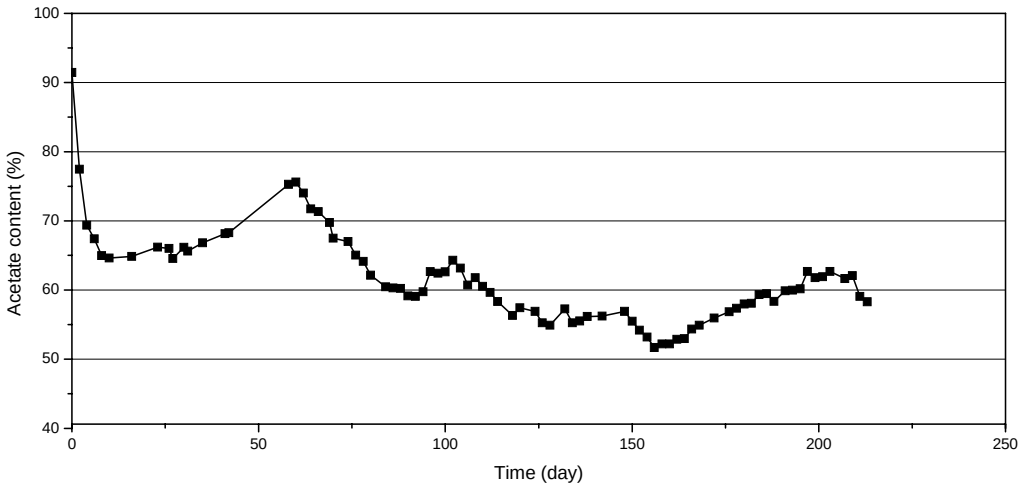


Figure 8-4. Acetate content for hot-lime-water-treated bagasse Fermentation Train CC (calcium carbonate, fresh solid 12 g, fresh liquid 100 mL, and constant cake weight 300 g).

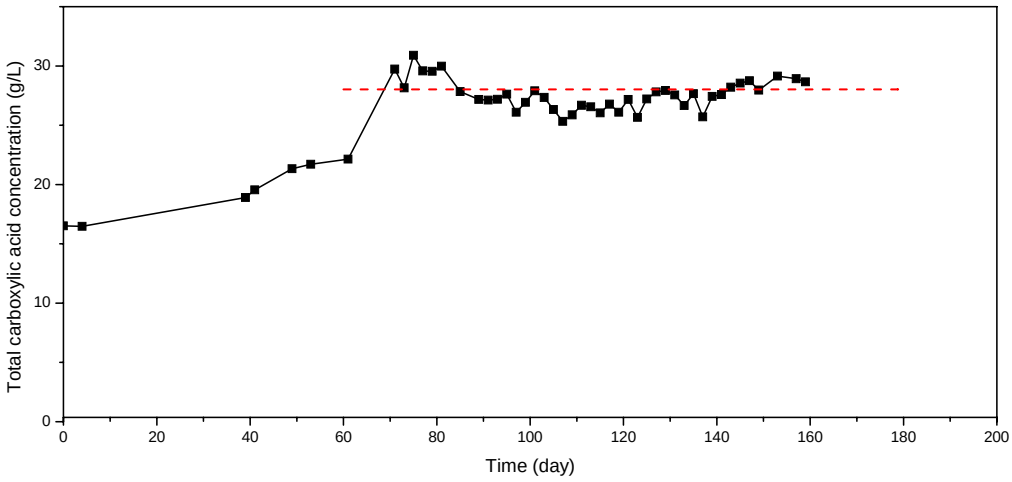


Figure 8-5. Total acid concentration for hot-lime-water-treated bagasse Fermentation Train CE (calcium carbonate, fresh solid 16 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (28.02 g/L).

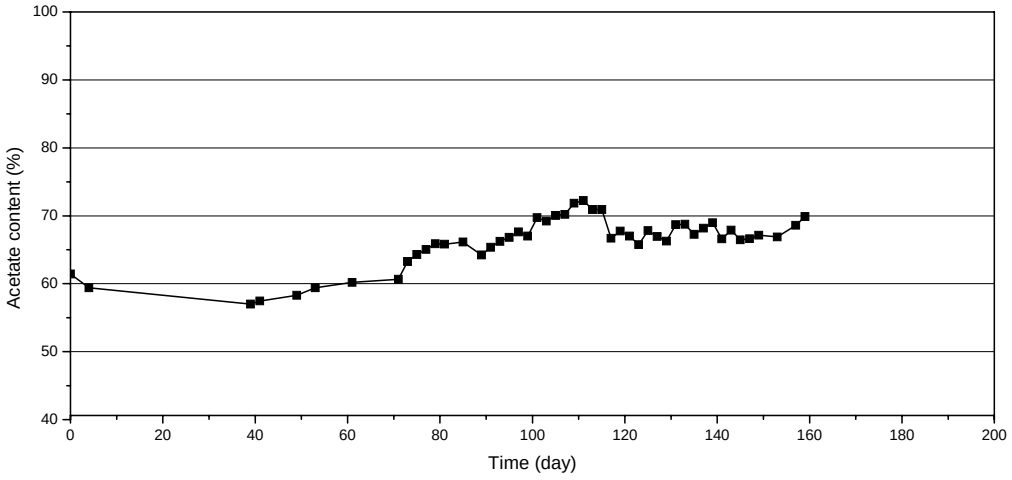


Figure 8-6. Acetate content for hot-lime-water-treated bagasse Fermentation Train CE (calcium carbonate, fresh solid 16 g, fresh liquid 100 mL, and constant cake weight 300 g).

8.2.4 Train CF

Four batch fermentations were initiated by evenly distributing the harvested solids and liquids from Train CC. Each batch fermentations was started by adding solid cake (80 g) from Train CC, residual liquid (108 mL) from Train CC, hot-lime-water-treated bagasse (32 g), chicken manure (8 g), calcium carbonate (3 g), nutrient mixture (0.2 g), urea (0.1 g), anaerobic water (150 mL), and 120 μL iodoform solution (20 g/L of iodoform dissolved in ethanol). The residual liquid and residual solids from Train CC provided the initial microorganisms to Train CF. On each transfer with Train CF, hot-lime-water-treated bagasse (9.6 g), chicken manure (2.4 g), nutrients (0.2 g), calcium carbonate (2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g), calcium carbonate (2 g), and iodoform (60 μL) were added to F2, F3, and F4. Fresh anaerobic water (100 mL) was added to F4 on each transfer. Urea (0.1 g) was added as a nitrogen source if the pH in the fermentation broth was below 6.0. The total acid concentration profile and acetate content profile are illustrated in Figures 8-7 and 8-8.

8.2.5 Summary of calcium carbonate buffered fermentations

Table 8-1 summarizes the operating conditions for fermentation trains using calcium carbonate buffers, whereas Table 8-2 shows the results for these countercurrent fermentations. Figure 8-9 lists the mass balance closures for these fermentations.

The highest acid productivity of 0.79 g/(L·day) occurred at a concentration of 21.49 g/L in Fermentation Train CF (LRT = 27.27 day and VSLR = 4.85 g/(L·day)). Fermentation Train CA (LRT = 25.85 day and VSLR = 3.26 g/(L·day)) with a concentration of 15.51 g/L had the highest conversion (0.59 g VS digested/g VS fed) and highest yield (0.18 g total acids/g VS fed). Fermentation Train CA had the highest conversion and yield because it had the lowest VSLR, which made more complete use of the biomass. The highest selectivity of 0.41 g total acids/g VS digested was found in fermentation train CC (LRT = 28.07 day and VSLR = 4.50 g/(L·day)).

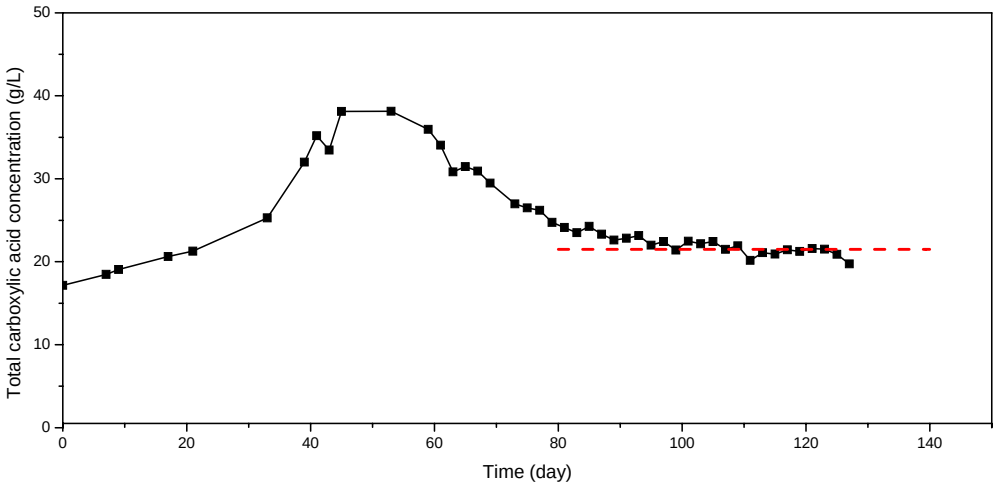


Figure 8-7. Total acid concentration for hot-lime-water-treated bagasse Fermentation Train CF (calcium carbonate, fresh solid 12 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (21.49 g/L).

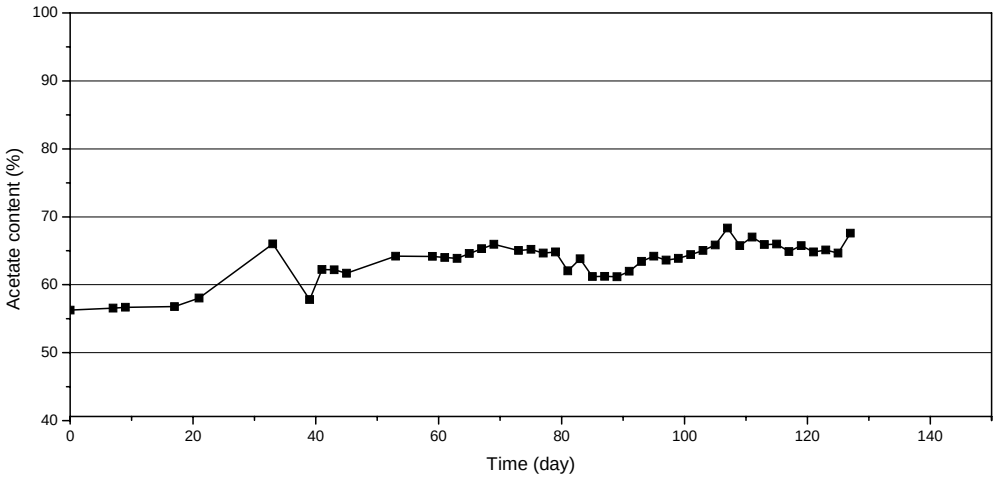


Figure 8-8. Total Acetate content for hot-lime-water-treated bagasse Fermentation Train CF (calcium carbonate, fresh solid 12 g, fresh liquid 100 mL, and constant cake weight 300 g).

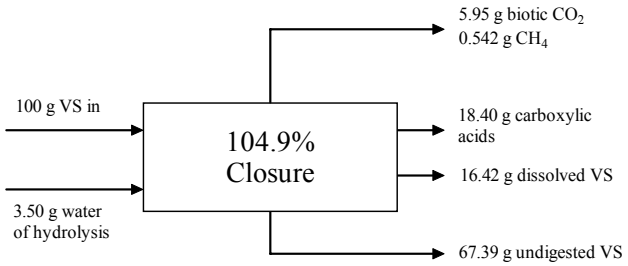
Table 8-1. Operating parameters for hot-lime-water-treated bagasse countercurrent fermentations using calcium carbonate.

Fermentation Trains	**CA**	**CC**	**CE**	**CF**
LRT (day)	25.85	28.07	42.26	27.27
VSLR (g VS/(L liquid in all fermentors·day))	3.26	4.50	6.24	4.85
VS feed at each transfer (g VS)	6.30	9.44	12.59	9.44
Solid feed at each transfer (g)	8.00	12.00	16.00	12.00
Treated bagasse (g)	6.40	9.60	12.80	9.60
Chicken manure (g)	1.60	2.40	3.20	2.40
Liquid fed to F4 at each transfer (L)	0.10	0.10	0.10	0.10
VS/liquid feed ratio (g VS/g liquid)	0.06	0.09	0.13	0.09
Liquid volume in all four fermentors (L)	0.97	1.05	1.01	0.97
Temperature (ºC)	55			
Frequency of transfer	Every two days			
Centrifuge Procedure	Single			
F_1 Retained weight (wet g)	292	288	284	280
F_2–F_4 Retained weight (wet g)	300	300	300	300
Iodoform addition rate (mg iodoform added/L liquid fed to F4)	24	24	24	24
Nutrients addition rate (g dry nutrients added/L liquid fed to F4)	2.0	2.0	2.0	2.0
Urea addition rate (g urea added/L liquid feed to F4)	0.0	0.0	0.0	0.0

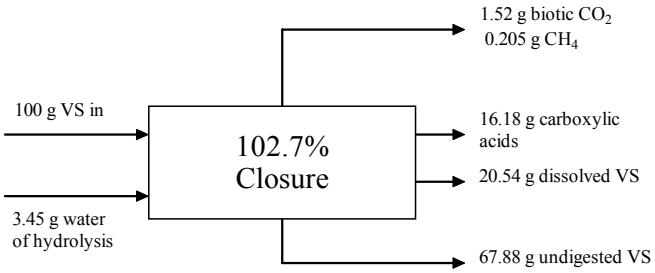
Table 8-2. Fermentation results for hot-lime-water-treated bagasse countercurrent fermentations using calcium carbonate.

Fermentation Trains	CA	CC	CE	CF
Average pH in all fermentors	6.03±0.27	6.07±0.26	5.88±0.16	5.88±0.09
Total carboxylic acid concentration (g/L)	15.51±0.71	20.46±0.86	28.02±0.78	21.49±0.65
Acetic acid (wt%)	59.05±1.82	60.50±2.13	67.44±1.02	65.53±1.13
Propionic acid (wt%)	2.74±1.06	1.40±0.23	1.23±0.08	1.48±0.14
Butyric acid (wt%)	33.90±1.45	34.74±1.95	27.19±0.84	27.86±1.05
valeric acid (wt%)	0.41±0.47	0.04±0.10	0.00±0.00	0.00±0.00
Caproic acid (wt%)	3.69±0.34	3.32±0.46	4.14±0.26	5.13±0.42
Heptanoic acid (wt%)	0.22±0.49	0.00±0.00	0.00±0.00	0.00±0.00
Conversion (g VS digested/g VS fed)	0.59	0.40	0.34	0.47
Yield (g total acids/g VS fed)	0.18	0.16	0.11	0.16
Selectivity (g total acids/g VS digested)	0.31	0.41	0.31	0.35
Total carboxylic acid productivity (g total acids/ (L liquid·day))	0.60	0.73	0.66	0.79
Methane productivity (g CH_4/(L liquid·day))	0.0177	0.0092	0.0083	0.0963
Mass balance closure (g VS out/g VS in)	1.049	1.027	0.989	1.054

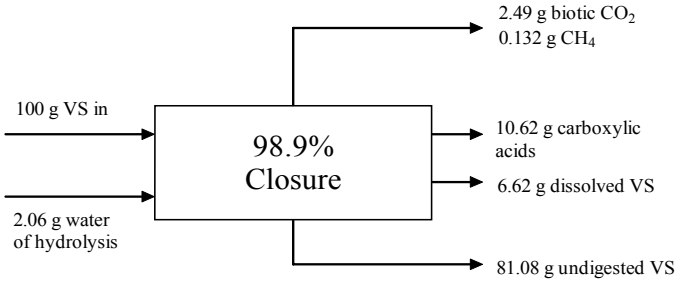
Note: All errors are ± 1 standard deviation.



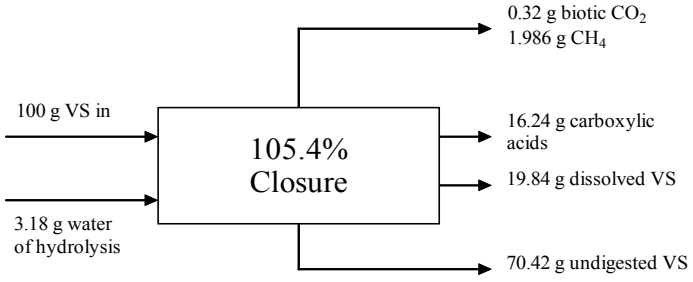
(a) For Fermentation CA.



(b) For Fermentation CC.



(c) For Fermentation CE.



(d) For Fermentation CF.

Figure 8-9. Mass balances for hot-lime-water-treated bagasse Fermentations CA, CC, CE, and CF.

8.3 Hot-lime-water-treated bagasse fermentation buffered by ammonium bicarbonate

A series of seven countercurrent fermentations were performed using ammonium bicarbonate as the pH buffer. No urea was used in ammonium bicarbonate buffered fermentations, because ammonium bicarbonate itself is a nitrogen source. The seven fermentation trains are Trains MA, MB, MC, MD, ME, MF, and MG. Trains MA, MB, and MC were the first continuous experiments with ammonium bicarbonate. The preset constant weight of solid cakes in these three trains was 200 g, whereas the constant weight of solid cake in the other trains was 300 g.

8.3.1 Train MA

Four batch fermentations were initiated by adding hot-lime-water-treated bagasse (32 g), chicken manure (8 g), ammonium bicarbonate (2 g), nutrient mixture (0.2 g), marine inocula (40 mL), anaerobic water (360 mL), and iodoform solution (120 μL). The marine inocula were taken from previous batch fermentation of hot-lime-water-treated bagasse and chicken manure using ammonium bicarbonate buffer. On each transfer with Train MA, hot-lime-water-treated bagasse (3.2 g), chicken manure (0.8 g), nutrients (0.2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g) and iodoform (60 μL) were added to F2, F3, and F4. Ammonium bicarbonate was added to control the pH in the fermentation broth around 7.0. The transfer of solids and liquids were performed as shown in Chapter VII. The transfer of liquids and solids was operated at a two-day interval for Train MA. Fresh anaerobic water (100 mL) was added to F4 on each transfer. The total acid concentration profile and acetate content profile are shown in Figures 8-10 and 8-11.

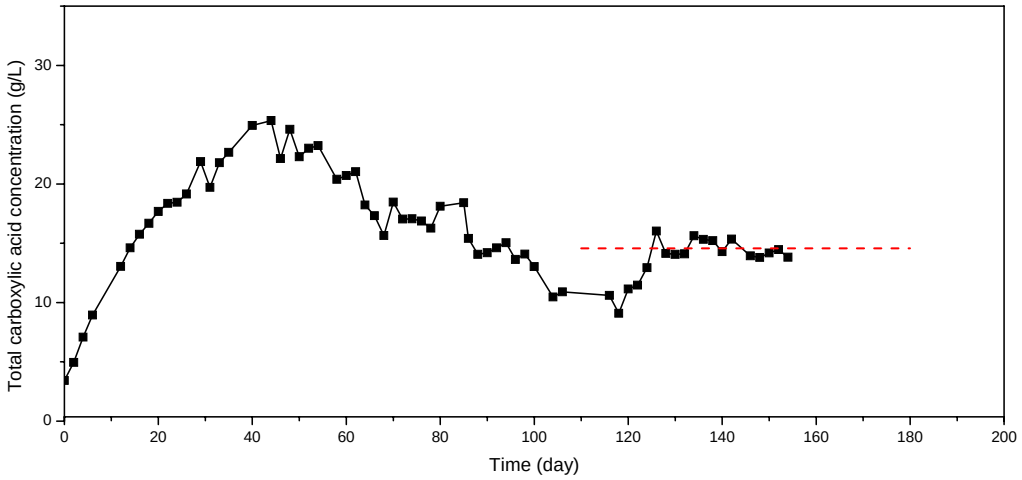


Figure 8-10. Total acid concentration for hot-lime-water-treated bagasse Fermentation Train MA (ammonium bicarbonate, fresh solid 4 g, fresh liquid 100 mL, and constant cake weight 200 g). Dash line indicates steady-state (14.57 g/L).

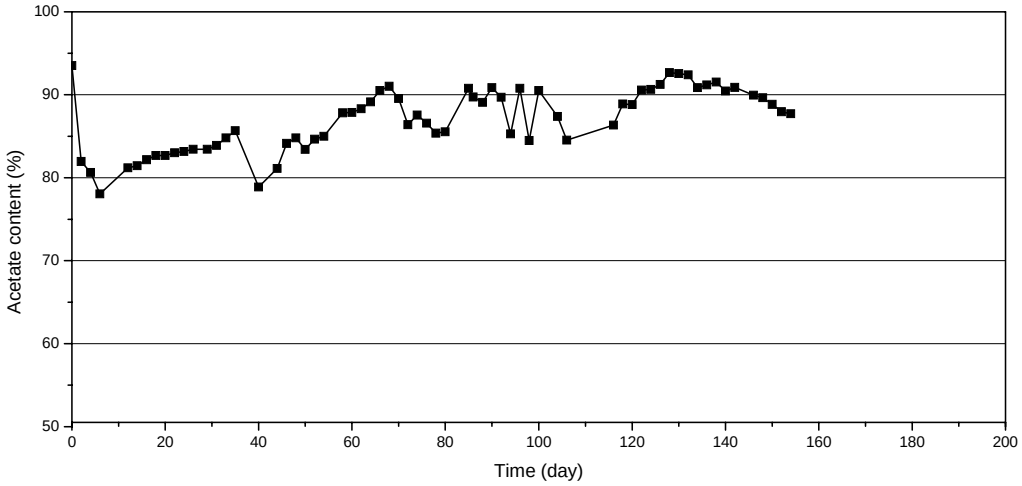


Figure 8-11. Acetate content for hot-lime-water-treated bagasse Fermentation Train MA (ammonium bicarbonate, fresh solid 4 g, fresh liquid 100 mL, and constant cake weight 200 g).

8.3.2 Train MB

Four batch fermentations were initiated by adding hot-lime-water-treated bagasse (32 g), chicken manure (8 g), ammonium bicarbonate (2 g), nutrient mixture (0.2 g), marine inocula (40 mL), anaerobic water (360 mL), and 120 μL iodoform solution (20 g/L of iodoform dissolved in ethanol). The marine inocula were taken from previous batch fermentation of hot-lime-water-treated bagasse and chicken manure using ammonium bicarbonate buffer. On each transfer with Train MB, hot-lime-water-treated bagasse (6.4 g), chicken manure (1.6 g), nutrients (0.2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g) and iodoform (60 μL) were added to F2, F3, and F4. Ammonium bicarbonate was added to control the pH in the fermentation broth around 7.0 (6.97–7.03). The transfer of solids and liquids was performed as shown in Chapter VII. The transfer of liquids and solids was operated at a two-day interval for Train MB. Fresh anaerobic water (100 mL) was added to F4 on each transfer. The total acid concentration profile and acetate content profile are shown in Figures 8-12 and 8-13.

Unfortunately, there was an experimental error on Day 242. Solid was added to F4 by mistake, and the liquid was added to F3. The train was nearly steady state at that time, but had to reestablish the stead-state. Train MB gained steady state again on Day 340.

The continuous operation time of over 350 days shows that anaerobic microorganisms from the marine source are adaptable to ammonium bicarbonate buffer and could produce stable carboxylic acids in a long-term operation. This information is very useful for pilot plant design, because stability is an important concern.

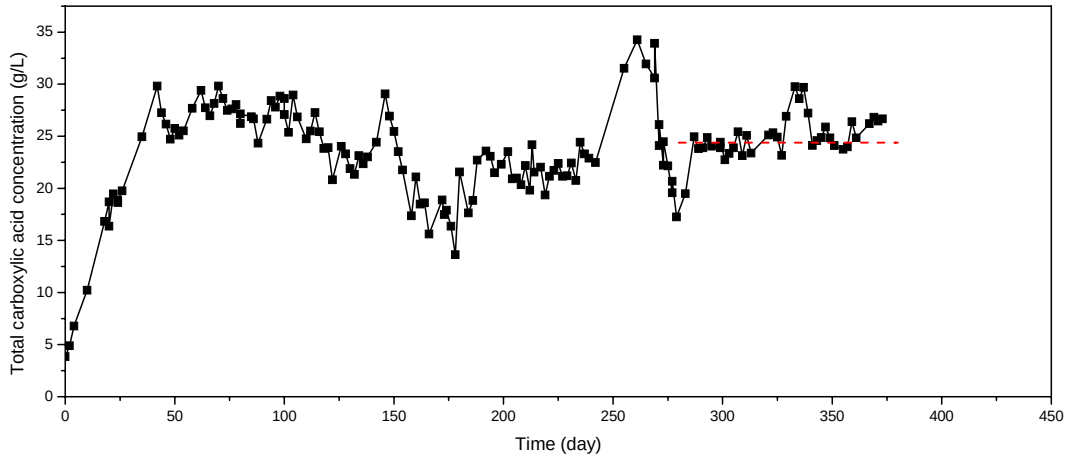


Figure 8-12. Total acid concentration for hot-lime-water-treated bagasse Fermentation Train MB (ammonium bicarbonate, fresh solid 8 g, fresh liquid 100 mL, and constant cake weight 200 g). Dash line indicates steady-state (24.40 g/L).

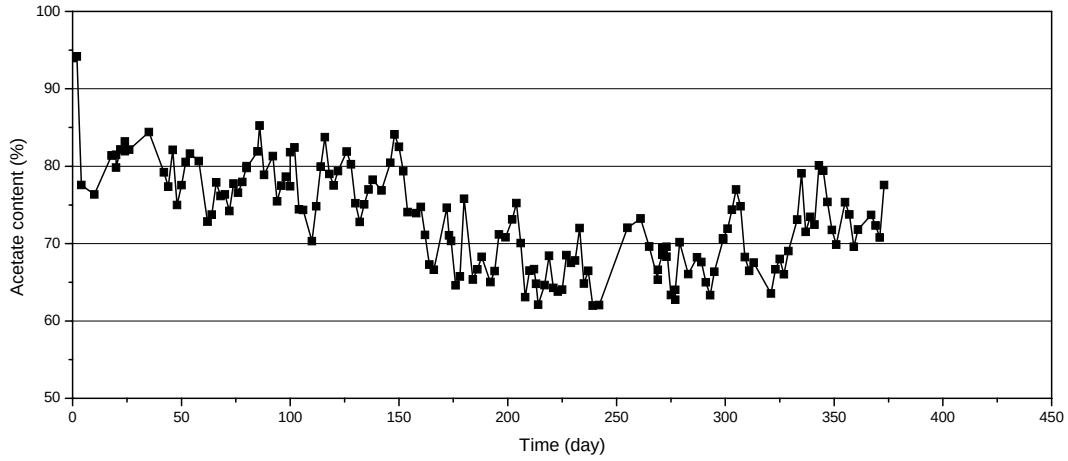


Figure 8-13. Acetate content for hot-lime-water-treated bagasse Fermentation Train MB (ammonium bicarbonate, fresh solid 8 g, fresh liquid 100 mL, and constant cake weight 200 g).

8.3.3 Train MC

Four batch fermentations were initiated by adding hot-lime-water-treated bagasse (32 g), chicken manure (8 g), ammonium bicarbonate (2 g), nutrient mixture (0.2 g), marine inocula (40 mL), anaerobic water (360 mL), and 120 μL iodoform solution (20 g/L of iodoform dissolved in ethanol). The marine inocula were taken from a previous batch of hot-lime-water-treated bagasse and chicken manure fermentations with ammonium bicarbonate buffer. On each transfer with Train MC, hot-lime-water-treated bagasse (6.4 g), chicken manure (1.6 g), nutrients (0.2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g) and iodoform (60 μL) were added to F2, F3, and F4. Ammonium bicarbonate was added to control the pH in the fermentation broth around 7.0 (6.97–7.03). The transfer of solids and liquids was performed as shown in Chapter VII. The transfer of liquids and solids was operated at a two-day interval for Train MC. Fresh anaerobic water (150 mL) was added to F4 on each transfer. The total acid concentration profile and acetate content profile are shown in Figures 8-14 and 8-15.

8.3.4 Train MD

Four batch fermentations were initiated by adding hot-lime-water-treated bagasse (32 g), chicken manure (8 g), ammonium bicarbonate (2 g), nutrient mixture (0.2 g), marine inocula (40 mL), anaerobic water (360 mL), and 120 μL iodoform solution (20 g/L of iodoform dissolved in ethanol). The marine inocula were taken from a previous batch of hot-lime-water-treated bagasse and chicken manure fermentations with ammonium bicarbonate buffer. On each transfer with Train MD, hot-lime-water-treated bagasse (9.6 g), chicken manure (2.4 g), nutrients (0.2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g) and iodoform (60 μL) were added to F2, F3, and F4. Ammonium bicarbonate was added to control the pH in the fermentation broth around 7.0 (6.97–7.03). The transfer of solids and liquids was performed as shown in Chapter VII. Fresh anaerobic water (100 mL) was added to F4 on each transfer. The total acid concentration profile and acetate content profile are shown in Figures 8-16 and 8-17.

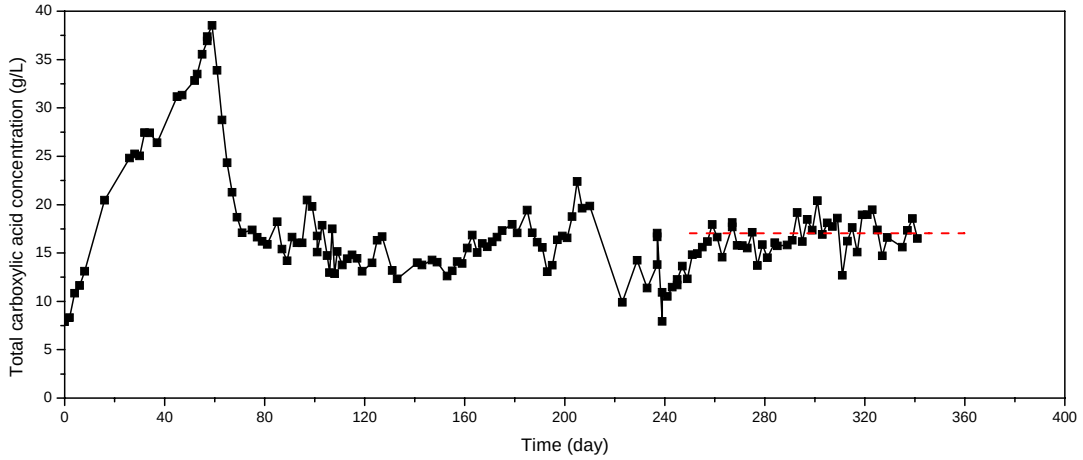


Figure 8-14. Total acid concentration for hot-lime-water-treated bagasse Fermentation Train MC (ammonium bicarbonate, fresh solid 8 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (17.06 g/L).

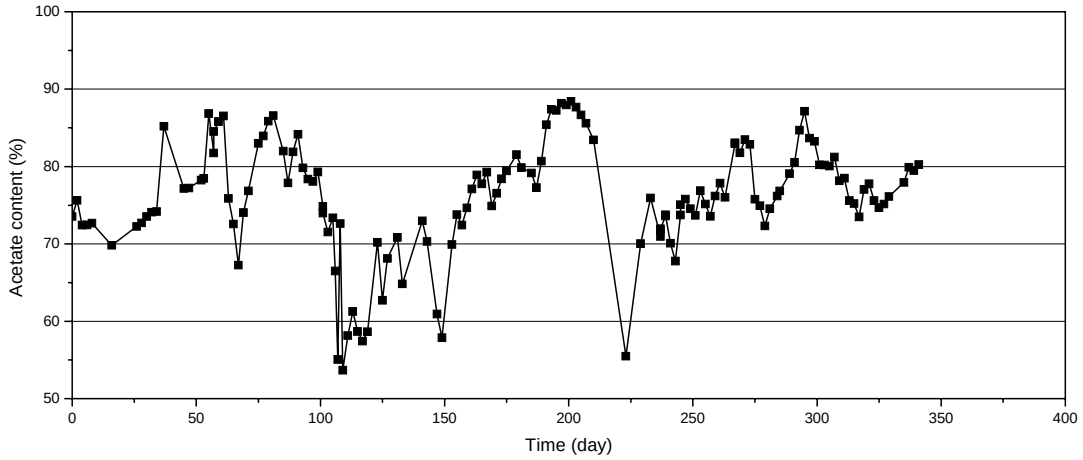


Figure 8-15. Acetate content for hot-lime-water-treated bagasse Fermentation Train MC (ammonium bicarbonate, fresh solid 8 g, fresh liquid 100 mL, and constant cake weight 300 g).

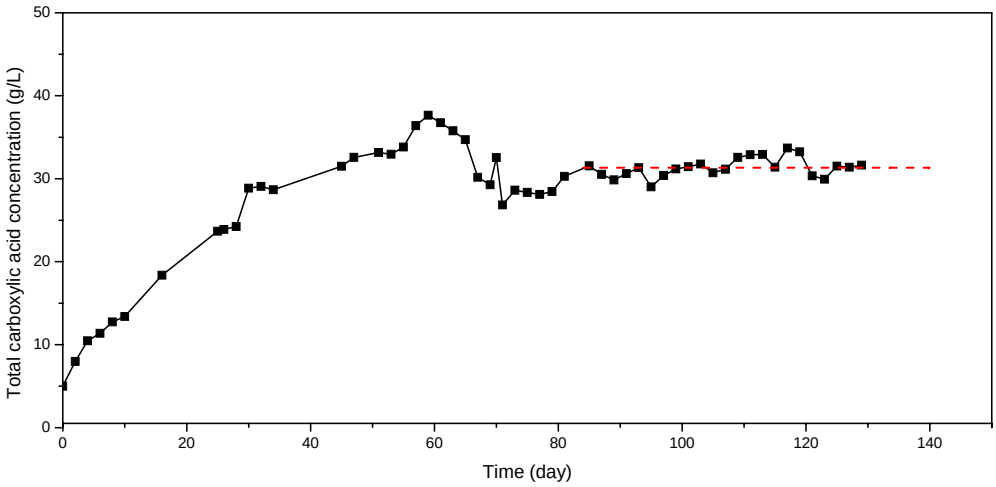


Figure 8-16. Total acid concentration for hot-lime-water-treated bagasse Fermentation Train MD (ammonium bicarbonate, fresh solid 12 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (31.34 g/L).

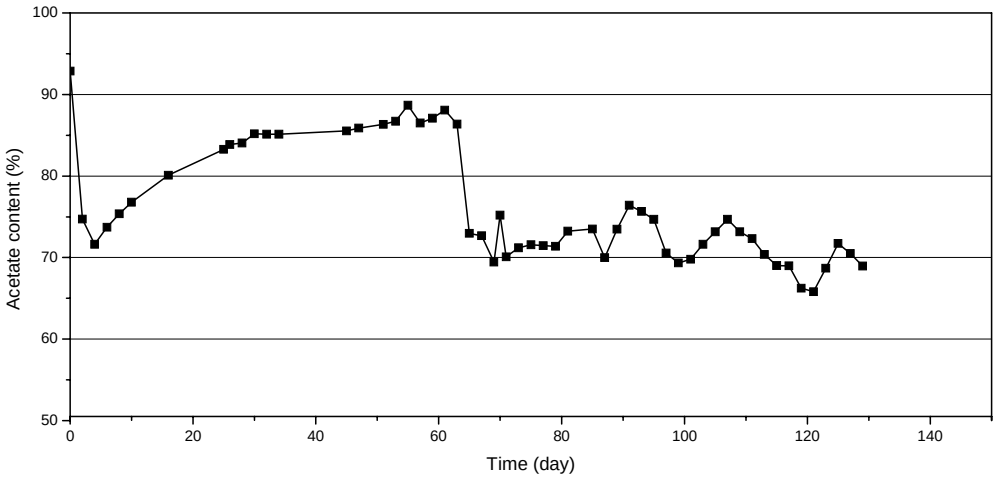


Figure 8-17. Acetate content for hot-lime-water-treated bagasse Fermentation Train MD (ammonium bicarbonate, fresh solid 12 g, fresh liquid 100 mL, and constant cake weight 300 g).

8.3.5 Train ME

Four batch fermentations were initiated by adding hot-lime-water-treated bagasse (32 g), chicken manure (8 g), ammonium bicarbonate (2 g), nutrient mixture (0.2 g), marine inocula (40 mL), anaerobic water (360 mL), and 120 μL iodoform solution (20 g/L of iodoform dissolved in ethanol). The marine inocula were taken from a previous batch of hot-lime-water-treated bagasse and chicken manure fermentations with ammonium bicarbonate buffer. On each transfer with Train ME, hot-lime-water-treated bagasse (12.8 g), chicken manure (3.2 g), nutrients (0.2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g) and iodoform (60 μL) were added to F2, F3, and F4. Ammonium bicarbonate was added to control the pH in the fermentation broth around 7.0 (6.97–7.03). The transfer of solids and liquids was performed as shown in Chapter VII. The transfer of liquids and solids was operated at a two-day interval for Train ME. Fresh anaerobic water (100 mL) was added to F4 on each transfer. The total acid concentration profile and acetate content profile are shown in Figures 8-18 and 8-19.

8.3.6 Train MF

Train MF was a continuation of Train ME, but operated with a different solid feed ratio. The residual solids and residual liquids in ME train were even distributed into 4 identical fermentations. Each batch fermentations was started by adding solid cake (80 g) from Train ME, residual liquid (80 mL) from Train ME, hot-lime-water-treated bagasse (19.2 g), chicken manure (4.8 g), nutrient mixture (0.2 g), anaerobic water (200 mL), and 120 μL of iodoform solution. There is a 12-day batch stage for Train MF. The countercurrent transfer was initiated on Day 12. On each transfer with Train MF, hot-lime-water-treated bagasse (19.2 g), chicken manure (4.8 g), nutrients (0.2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g) and iodoform (60 μL) were added to F2, F3, and F4. Fresh anaerobic water (100 mL) was added to F4 on each transfer. The total acid concentration profile and acetate content profile are illustrated in Figures 8-20 and 8-21.

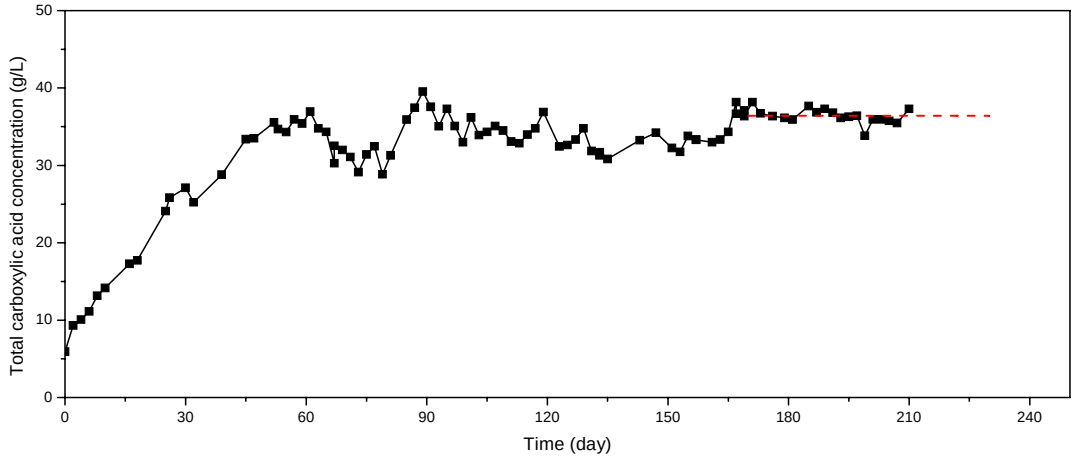


Figure 8-18. Total acid concentration for hot-lime-water-treated bagasse Fermentation Train ME (ammonium bicarbonate, fresh solid 16 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (36.43 g/L).

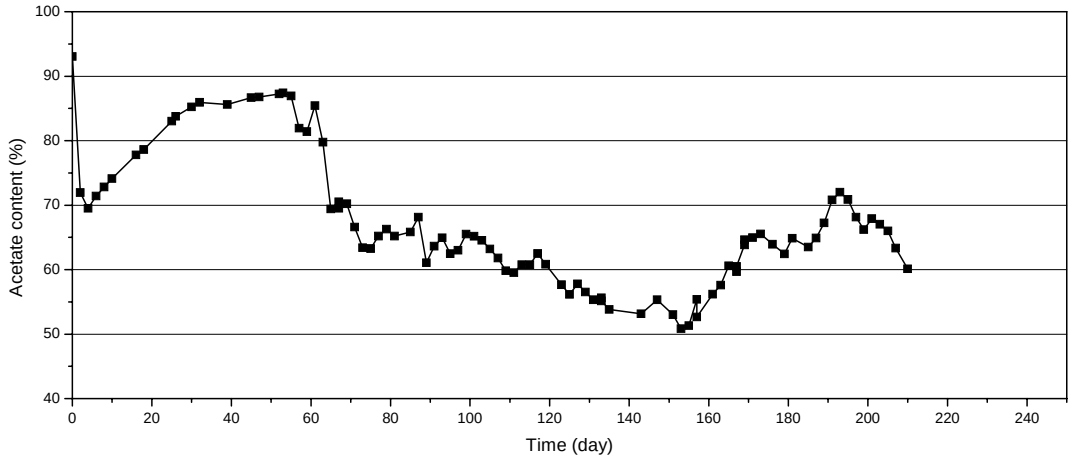


Figure 8-19. Acetate content for hot-lime-water-treated bagasse Fermentation Train ME (ammonium bicarbonate, fresh solid 16 g, fresh liquid 100 mL, and constant cake weight 300 g).

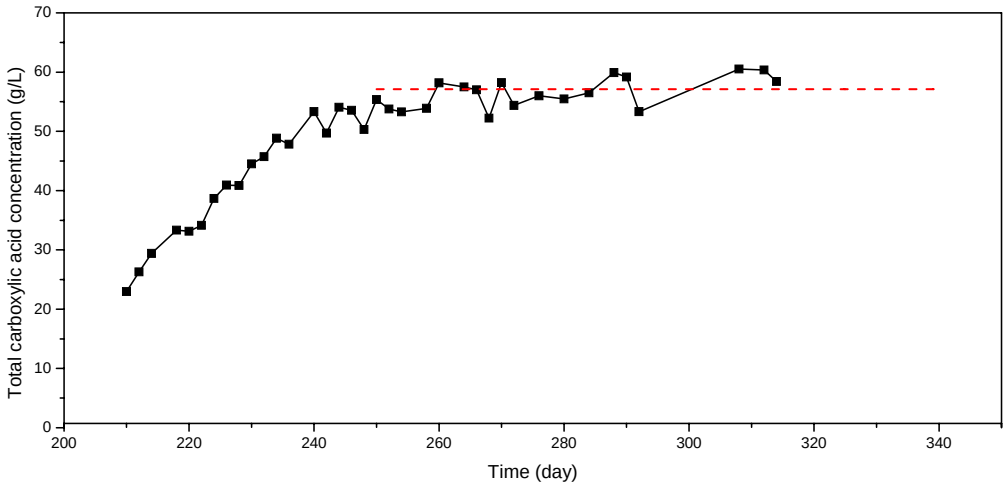


Figure 8-20. Total acid concentration for hot-lime-water-treated bagasse Fermentation Train MF (ammonium bicarbonate, fresh solid 24 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (57.14 g/L).

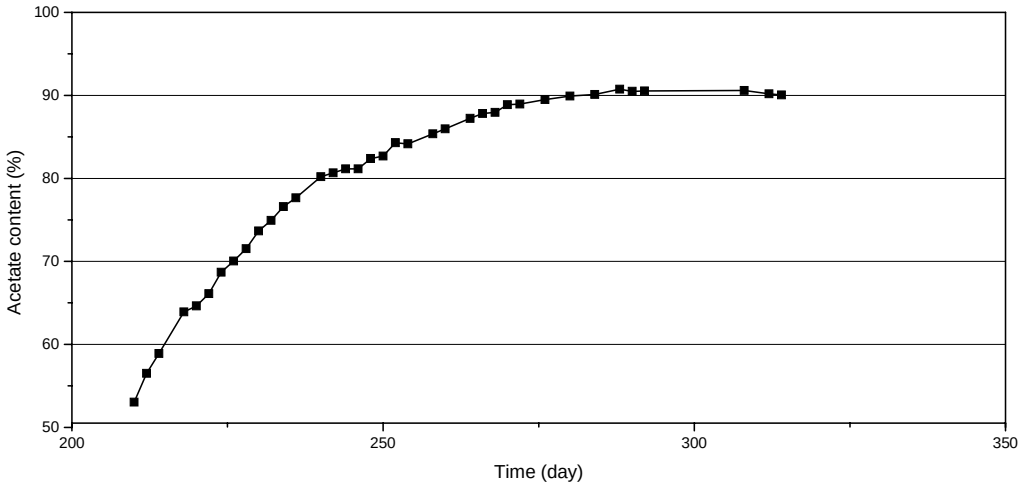


Figure 8-21. Acetate content for hot-lime-water-treated bagasse Fermentation Train MF (ammonium bicarbonate, fresh solid 24 g, fresh liquid 100 mL, and constant cake weight 300 g).

8.3.7 Train MG

Train MG was a continuation of Train MF, but operated with a different solid feed ratio (20 g fresh biomass to F1). Train MG did not redistribute the solids and liquids of Train MF. There was no batch stage for train MG. On each transfer with Train MG, hot-lime-water-treated bagasse (16.0 g), chicken manure (4.0 g), nutrients (0.2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g) and iodoform (60 μL) were added to F2, F3, and F4. The transfer of liquids and solids was operated at a two-day interval for Train MG. Fresh anaerobic water (100 mL) was added to F4 on each transfer. The total acid concentration profile and acetate content profile are illustrated in Figures 8-22 and 8-23.

8.3.8 Summary of ammonium bicarbonate buffered fermentations

Table 8-3 summarizes the operating conditions for fermentation trains using ammonium bicarbonate buffer, whereas Table 8-4 shows the results for these countercurrent fermentations. Figures 8-24 and 8-25 list the mass balance closures for these fermentations.

The highest acid productivity of 1.27 g/(L·day) occurred at a concentration of 24.40 g/L in Fermentation Train MB (LRT = 19.26 day and VSLR = 3.32 g/(L·day)). Fermentation Train MD (LRT = 26.56 day and VSLR = 4.31 g/(L·day)) with a concentration of 31.34 g/L had highest conversion (0.76 g VS digested/g VS fed) and yield (0.27 g total acids/g VS fed). Fermentation Train MD had the highest conversion among Trains MD, ME, MF, and MG, because it had the lowest VSLR, which made more complete use of the biomass. The highest selectivity of 0.55 g total acids/g VS digested was in fermentation train MA (LRT = 19.10 day and VSLR = 2.07 g/(L·day)).

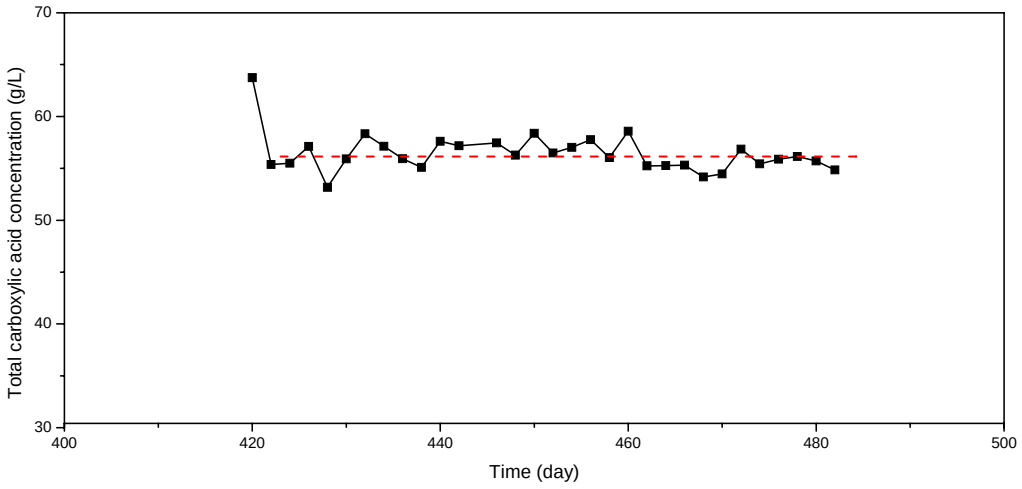


Figure 8-22. Total acid concentration for hot-lime-water-treated bagasse Fermentation Train MG (ammonium bicarbonate, fresh solid 20 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (56.14 g/L).

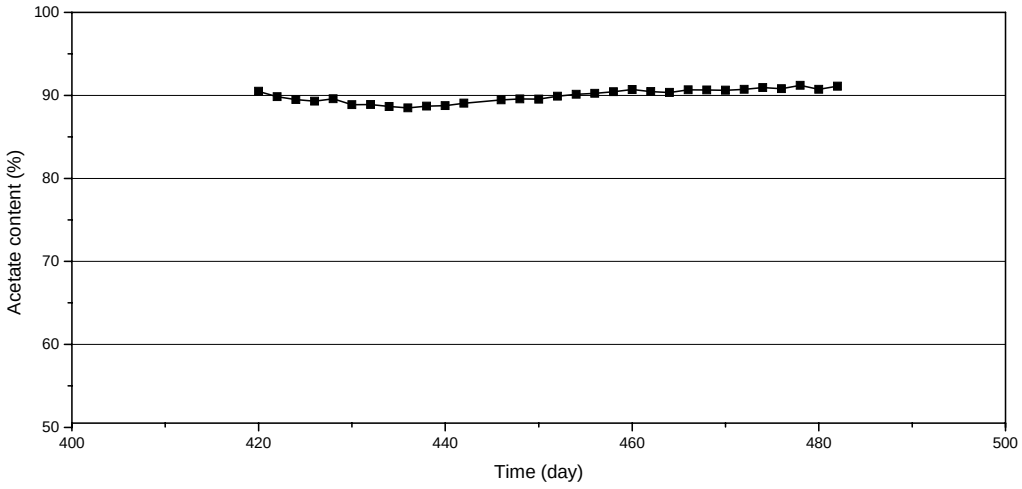


Figure 8-23. Acetate content for hot-lime-water-treated bagasse Fermentation Train MG (ammonium bicarbonate, fresh solid 20 g, fresh liquid 100 mL, and constant cake weight 300 g).

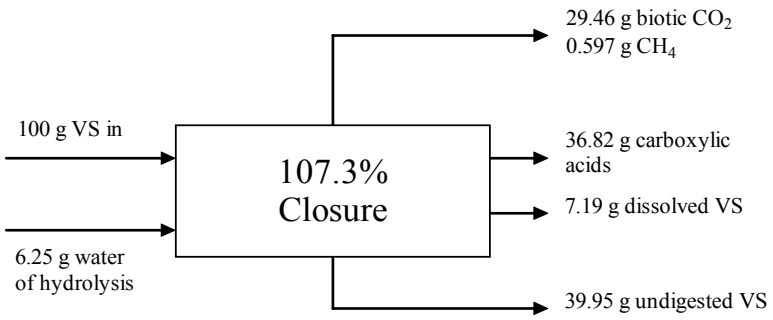
Table 8-3. Operating parameters for hot-lime-water-treated bagasse countercurrent fermentations using ammonium bicarbonate.

Fermentation Trains	MA	MB	MC	MD	ME	MF	MG
LRT (day)	19.10	19.26	14.29	26.56	31.78	131.35	44.72
VSLR (g VS/L liquid in all fermentors·day)	2.07	4.03	3.32	4.31	5.50	8.96	6.79
VS feed at each transfer (g VS)	3.15	6.30	6.30	9.44	12.59	18.89	15.74
Solid feed at each transfer (g)	4.00	8.00	8.00	12.00	16.00	24.00	20.00
Treated bagasse (g)	3.20	6.40	6.40	9.60	12.80	19.20	16.00
Chicken manure (g)	0.80	1.60	1.60	2.40	3.20	4.80	4.00
Liquid fed to F4 at each transfer (L)	0.10	0.10	0.15	0.10	0.10	0.10	0.10
VS/liquid feed ratio (g VS/g liquid)	0.03	0.06	0.04	0.09	0.13	0.19	0.16
Liquid volume in all four fermentors (L)	0.76	0.78	0.95	1.10	1.14	1.05	1.16
Temperature (ºC)	55						
Frequency of transfer	Every two days						
Centrifuge Procedure	Single						
F_1 Retained weight (wet g)	196	192	192	288	284	276	280
F_2–F_4 Retained weight (wet g)	200	200	200	300	300	300	300
Iodoform addition rate (mg iodoform /L liquid fed to F4)	24	24	24	24	24	24	24
Nutrients addition rate (g dry nutrients/L liquid fed to F4)	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Urea addition rate (g urea added/L liquid feed to F4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0

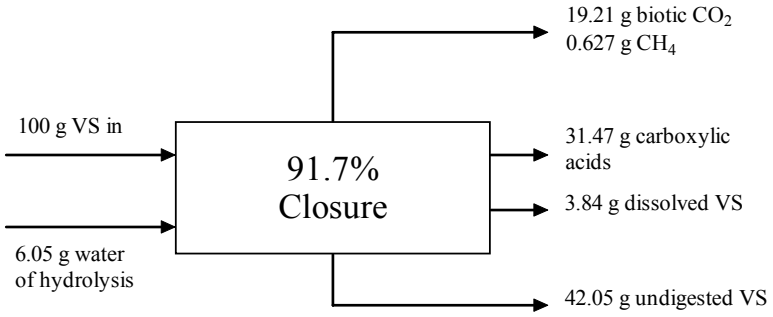
Table 8-4. Fermentation results for hot-lime-water-treated bagasse countercurrent fermentations using ammonium bicarbonate.

Fermentation Trains	MA	MB	MC	MD	ME	MF	MG
pH (F1)	7.06±0.60	6.71±0.41	6.76±0.45	6.88±0.34	6.87±0.35	6.97±0.40	6.76±0.28
Total carboxylic acid concentration (g/L)	14.57±0.66	24.40±1.39	17.06±1.74	31.34±1.18	36.43±0.92	57.14±2.51	56.14±1.23
Acetic acid (wt%)	90.56±1.41	73.87±3.46	77.57±2.31	71.14±2.84	65.92±2.98	89.26±1.43	90.28±0.74
Propionic acid (wt%)	1.87±0.30	2.90±0.66	2.48±0.23	3.50±0.38	2.38±0.17	2.25±0.12	2.61±0.07
Butyric acid (wt%)	6.94±1.71	22.86±3.82	19.51±2.52	24.59±3.06	31.12±3.03	7.99±1.32	6.66±0.73
valeric acid (wt%)	0.63±0.38	0.37±0.24	0.44±0.42	0.76±0.13	0.54±0.08	0.26±0.02	0.25±0.02
Caproic acid (wt%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.04	0.00±0.00	0.00±0.00
Heptanoic acid (wt%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.03±0.18	0.24±0.07	0.20±0.06
Conversion (g VS digested/g VS fed)	0.67	0.62	0.66	0.76	0.66	0.20	0.44
Yield (g total acids/g VS fed)	0.37	0.31	0.36	0.27	0.21	0.05	0.18
Selectivity (g total acids/g VS digested)	0.55	0.51	0.54	0.36	0.32	0.25	0.42
Total carboxylic acid productivity (g total acids/ (L liquid·day))	0.76	1.27	1.19	1.18	1.15	0.44	1.26
Methane productivity (g CH_4/(L liquid·day))	0.0124	0.0252	0.0687	0.0326	0.0135	0.0188	0.0253
Mass balance closure (g VS out/g VS in)	1.073	0.917	1.098	0.950	0.893	0.942	0.920

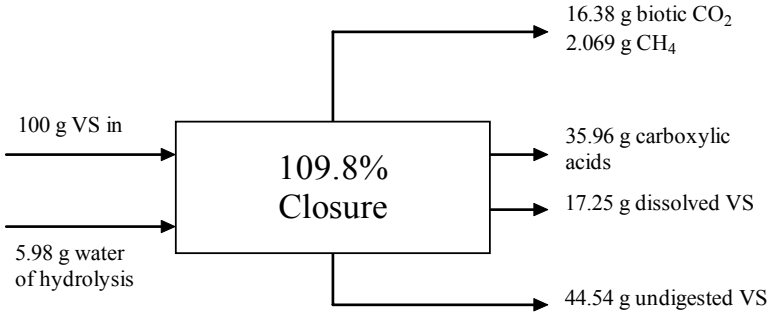
Note: All errors are ± 1 standard deviation.



(a) For Fermentation MA.

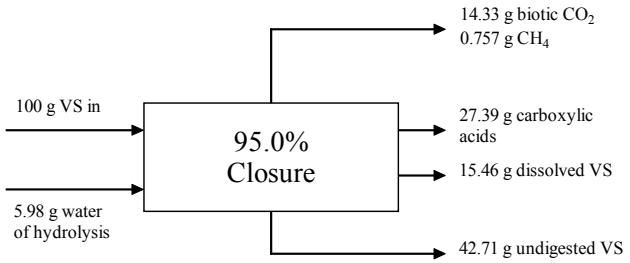


(b) For Fermentation MB.

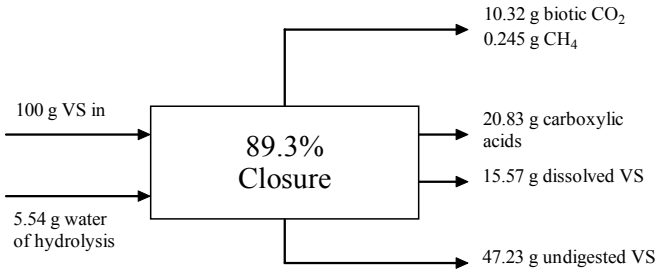


(c) For Fermentation MC.

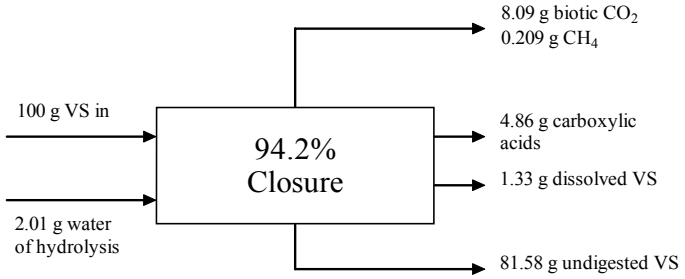
Figure 8-24. Mass balances for hot-lime-water-treated bagasse Fermentations MA, MB, and MC.



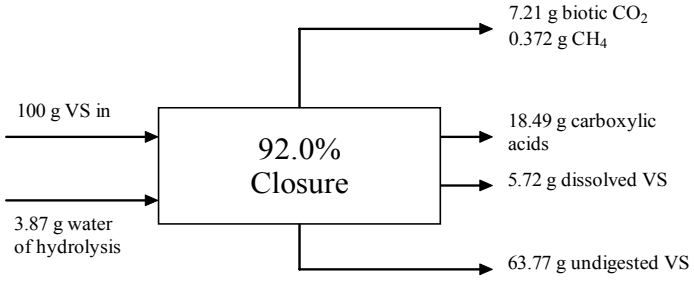
(e) For Fermentation MD.



(f) For Fermentation ME.



(g) For Fermentation MF.



(h) For Fermentation MG.

Figure 8-25. Mass balances for hot-lime-water-treated bagasse Fermentations MD, ME, MF, and MG.

8.4. CPDM prediction

8.4.1 Hot-lime-water-treated bagasse/chicken manure with calcium carbonate

Batch experiments with 80 wt% hot-lime-water-treated bagasse/20 wt% chicken manure were done to obtain model parameters for CPDM method, as mentioned in Chapter VII. Sugarcane bagasse was treated with lime for 2 h following the procedure in Appendix A. The marine inoculum for these fermentations was taken from countercurrent Trains CF running with the same hot-lime-water-treated bagasse, so the microorganisms were already adapted to the substrate. Calcium carbonate was used to adjust the pH. Liquid samples from the fermentation were analyzed for carboxylic acids. Carboxylic acid concentrations were converted to acetic acid equivalents (Aceq) using Equations 7-11 and 7-12. The Aceq concentrations for the five hot-lime-water-treated bagasse/chicken manure batch experiments are shown in Figures 8-26 to 8-30. The smooth lines are the predicted Aceq. Values of the fitted parameters *a*, *b*, and *c* for Equation 7-13 are presented in Table 8-5.

Table 8-5. Values of the parameters *a*, *b*, and *c* fitted by least squares analysis (lime-treated bagasse/chicken manure with calcium carbonate).

Substrate Concentration (g/L)	*a* (g /L liquid)	*b* (g /(L liquid·d))	*c* (d^{-1})
40	6.93	0.54	0.42
70	7.70	1.03	0.14
100	8.48	1.23	0.08
100+ (a)	26.17	1.02	0.14
100+ (b)	24.23	1.72	0.24

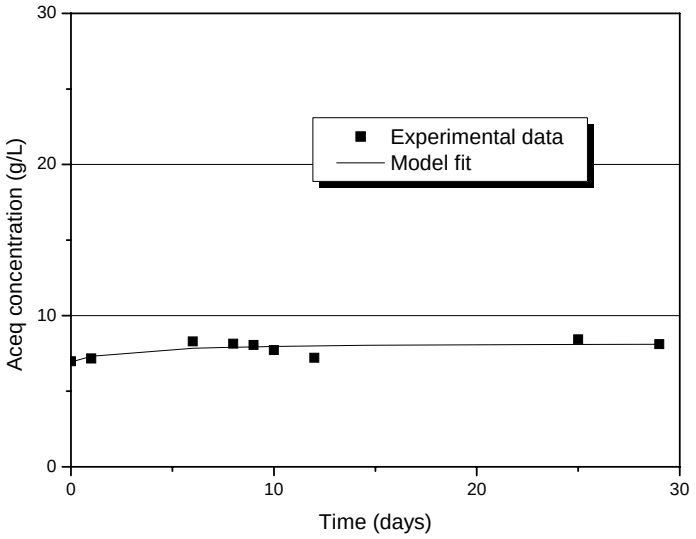


Figure 8-26. Aceq concentration for hot-lime-water-treated bagasse/chicken manure batch fermentation at 40 g substrate /L liquid with calcium carbonate.

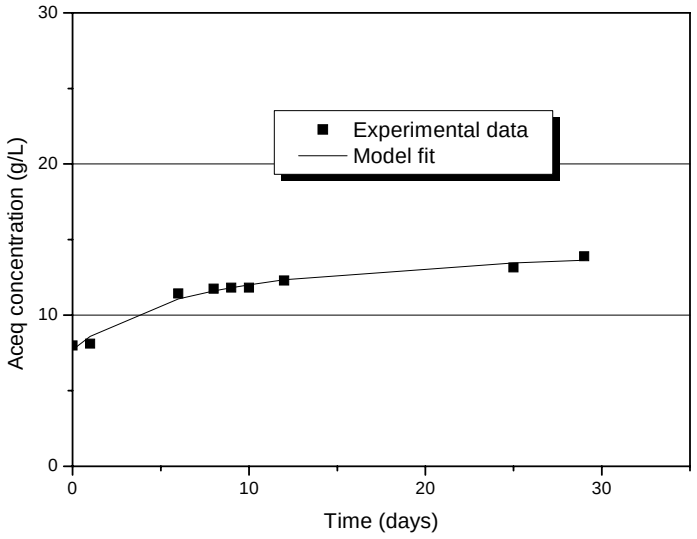


Figure 8-27. Aceq concentration for hot-lime-water-treated bagasse/chicken manure batch fermentation at 70 g substrate /L liquid with calcium carbonate.

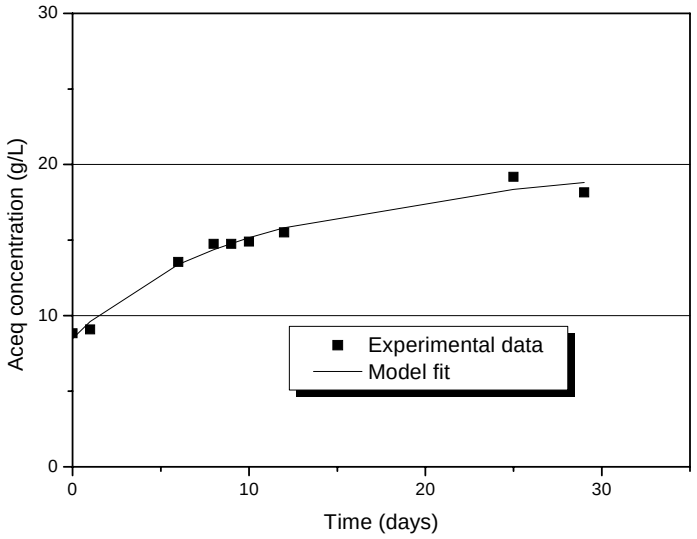


Figure 8-28. Aceq concentration for hot-lime-water-treated bagasse/chicken manure batch fermentation at 100 g substrate /L liquid with calcium carbonate.

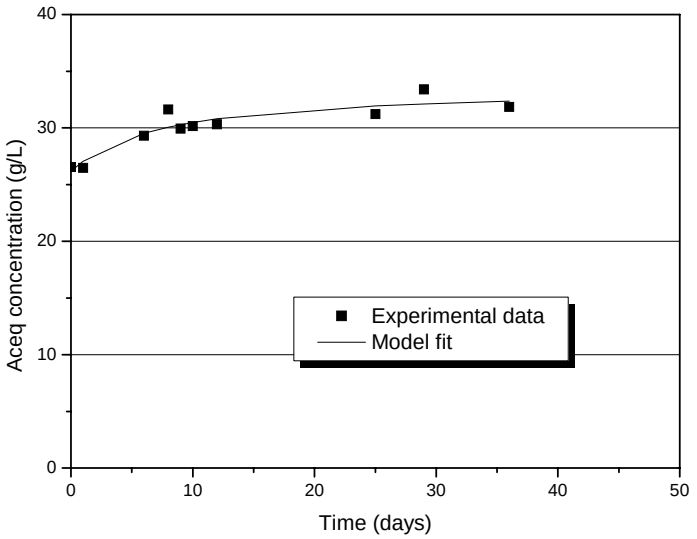


Figure 8-29. Aceq concentration for hot-lime-water-treated bagasse/chicken manure at 100 g substrate + acids (a)/L liquid with calcium carbonate.

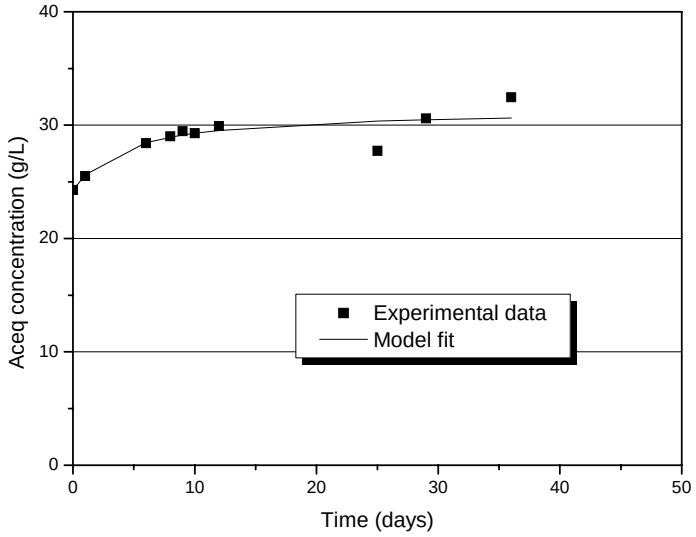


Figure 8-30. Aceq concentration for hot-lime-water-treated bagasse/chicken manure batch fermentor at 100 g substrate + acids (b)/L liquid with calcium carbonate.

The reaction rate and specific reaction rate for batch fermentations were calculated by using Equations 7-15 and 7-16. Conversion was calculated with the experimental acetic acid equivalents using Equation 7-17. Parameters *e*, *f*, *g*, and *h* present in the predicted rate equation (Equation 8-1) were calculated by nonlinear regression (*Systat Sigmaplot 10.0*). Figure 8-31 compares the predicted specific rate with the experimental specific rate. The specific rate equation for the 80 wt% hot-lime-water-treated bagasse/20 wt% chicken manure fermentation with calcium carbonate follows:

$$\hat{r}_{\text{pred}} = \frac{0.49\,(1-x)^{3.28}}{1+3.22(\phi \cdot \text{Aceq})^{0.95}} \tag{8-1}$$

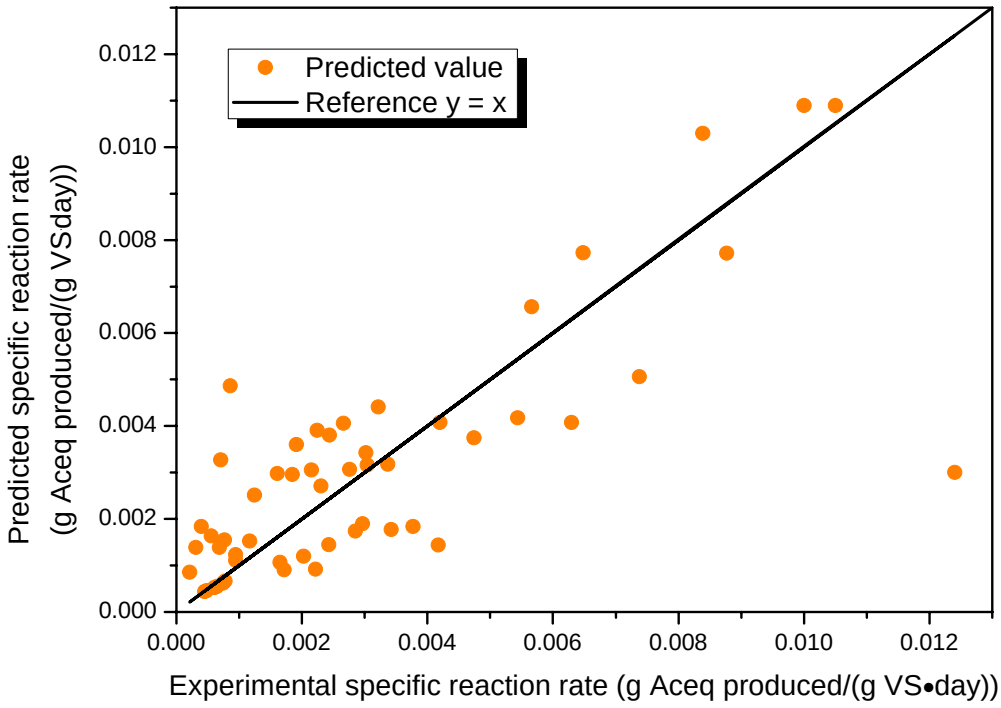


Figure 8-31. The experimental value and the CPDM prediction value for the specific reaction rate in five batch hot-lime-water-treated bagasse/chicken manure fermentations with calcium carbonate.

Table 8-6. Parameter constant values in CPDM for hot-lime-water-treated bagasse/chicken manure fermentation system with calcium carbonate.

Parameter constant	Value
Holdup (g liquid/g VS cake)	3.18
Moisture (g liquid/g solid feed)	0.03
Selectivity (g Aceq/g VS digested)	0.35
F1–F4 solid concentration (g VS/L)	124
F1–F4 liquid volume (L)	0.25
ϕ (g total acid/g Aceq)	0.85
e (g Aceq/(g VS·d))	0.49
f (dimensionless)	3.28
g (L/g total acid)$^{1/h}$	3.22
h (dimensionless)	0.95

Table 8-6 lists the system-specific variables required by the CPDM prediction, whereas Table 8-7 compares the experimental total carboxylic acid concentration and conversion to the CPDM prediction. As shown in Table 8-7, the total carboxylic acid concentrations from experiments agreed well with the CPDM predicted values, with an average absolute error of 9.98%. Substrate conversions for experimental and predicted conditions were very close, with an average absolute error of 7.39%.

Table 8-7. Comparison of experimental and predicted carboxylic acid concentration for hot-lime-water-treated bagasse/chicken manure fermentations with calcium carbonate.

	Train CA	**Train CC**	**Train CE**	**Train CF**	**Average[**] (%)**
Experimental carboxylic acid concentration (g/L)	15.51	20.46	28.02	21.49	
Predicted (CPDM) carboxylic acid concentration (g/L)	15.85	18.53	23.96	18.53	
Error[*] (%)	2.19	-9.45	-14.50	-13.79	9.98
Experimental conversion	0.59	0.48	0.34	0.47	
Predicted (CPDM) conversion	0.64	0.52	0.36	0.50	
Error[*] (%)	9.15	7.92	6.76	5.74	7.39

* Error (%) = ((Predicted value – Experimental value)/Experimental value) × 100

** Average errors are based on absolute value.

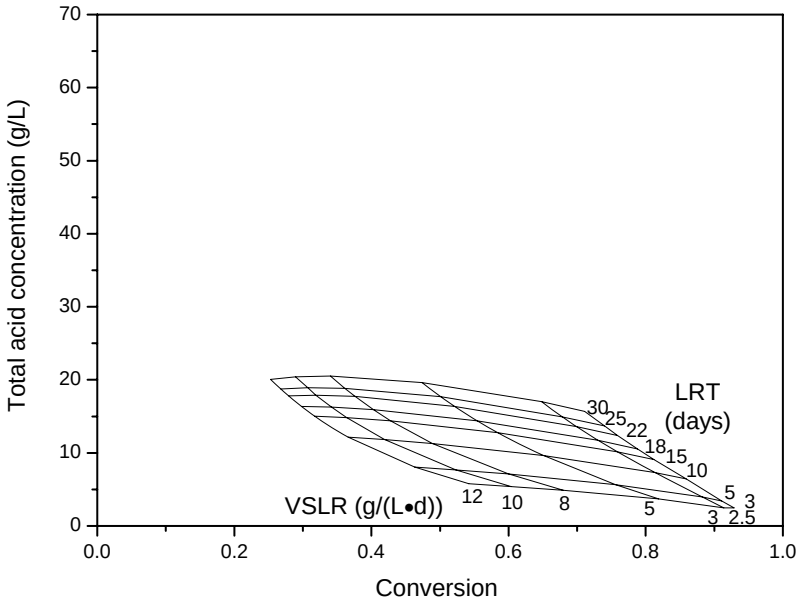


Figure 8-32. The CPDM "map" for 80 wt% hot-lime-water-treated bagasse/20 wt% chicken manure countercurrent fermentation with calcium carbonate buffer (124 g VS/L liquid).

Figure 8-32 shows the CPDM "map" for hot-lime-water-treated bagasse/chicken manure countercurrent fermentation with the single-centrifuge procedure at a fermentation solid concentration of 124 g VS/(L of liquid). The "map" predicts a total acid concentration of 20.53 g/L at LRT of 30 day, VSLR of 8 g/(L·d), and a conversion of 34.0%. At a VSLR of 2.5 g/(L·d) and LRT of 3 day, a total acid concentration of 2.47 g/L could be obtained at 92.9% conversion.

8.4.2 Hot-lime-water-treated bagasse/chicken manure with ammonium bicarbonate

Batch experiments with 80 wt% hot-lime-water-treated bagasse/20 wt% chicken manure were performed to obtain model parameters for CPDM method. Sugarcane bagasse was treated with lime for 2 h following the procedure in Appendix A. The marine inoculum for these fermentations was taken from countercurrent Train MG running with the same hot-lime-water-treated bagasse, so the microorganisms were already adapted to the substrate. Liquid samples from the fermentation were analyzed for carboxylic acids. Carboxylic acid concentrations were converted to Aceq concentrations using Equations 7-11 and 7-12. The Aceq concentrations for the five hot-lime-water-treated bagasse/chicken manure batch experiments are shown in Figures 8-33 to 8-37. The smooth lines are the predicted Aceq. Values of the fitted parameters *a*, *b*, and *c* for Equation 7-13 are presented in Table 8-8.

$$\text{Aceq} = a + \frac{bt}{1+ct} \tag{7-13}$$

Table 8-8. Values of the parameters *a*, *b*, and *c* fitted by least squares analysis (lime-treated bagasse/chicken manure with ammonium bicarbonate).

Substrate Concentration (g/L)	*a* (g /L liquid)	*b* (g /(L liquid·d))	*c* (d^{-1})
40	5.78	3.00	0.51
70	6.59	5.28	0.56
100	7.39	6.62	0.47
100+ (a)	24.46	2.17	0.16
100+ (b)	24.62	1.50	0.08

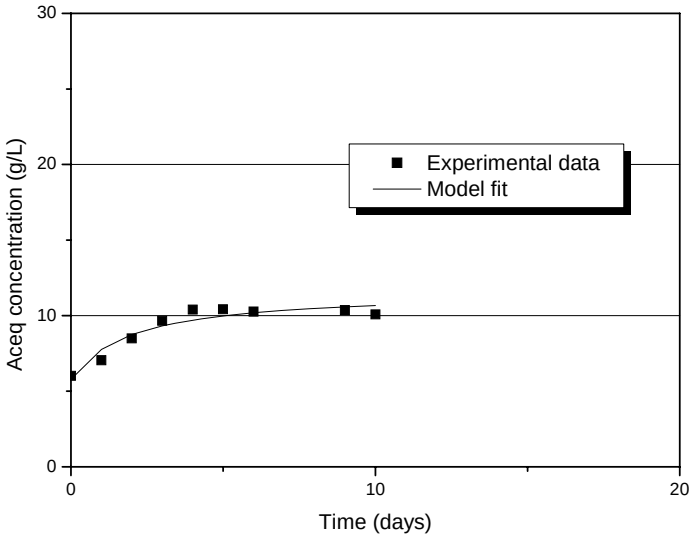


Figure 8-33. Aceq concentration for hot-lime-water-treated bagasse/chicken manure batch fermentor at 40 g substrate /L liquid with ammonium bicarbonate.

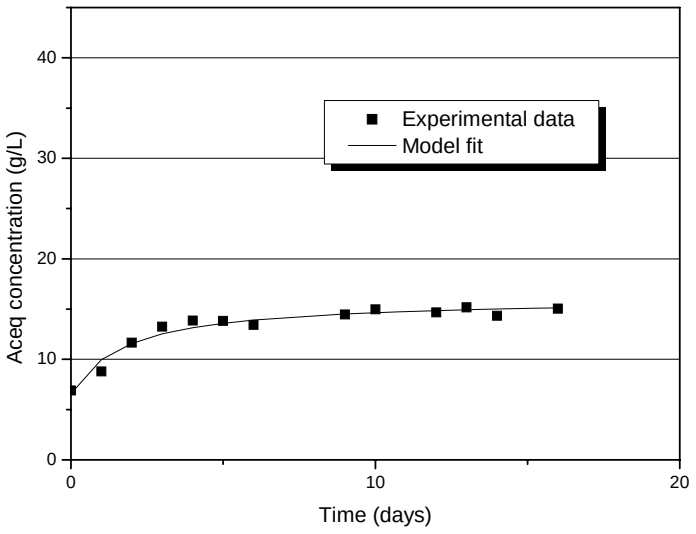


Figure 8-34. Aceq concentration for hot-lime-water-treated bagasse/chicken manure batch fermentor at 70 g substrate /L liquid with ammonium bicarbonate.

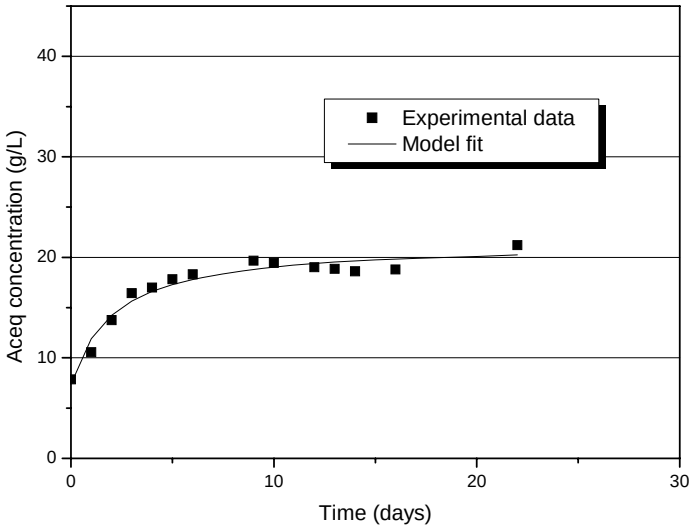


Figure 8-35. Aceq concentration for hot-lime-water-treated bagasse/chicken manure batch fermentor at 100 g substrate /L liquid with ammonium bicarbonate.

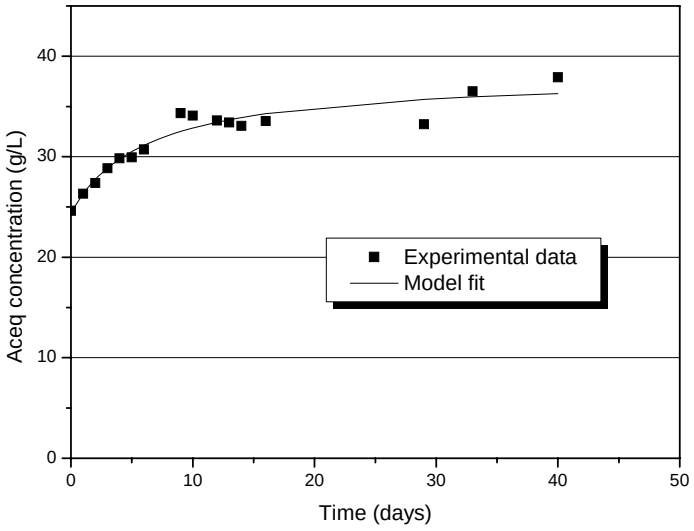


Figure 8-36. Aceq concentration for hot-lime-water-treated bagasse/chicken manure batch fermentor at 100 g substrate + acids (a)/L liquid with ammonium bicarbonate.

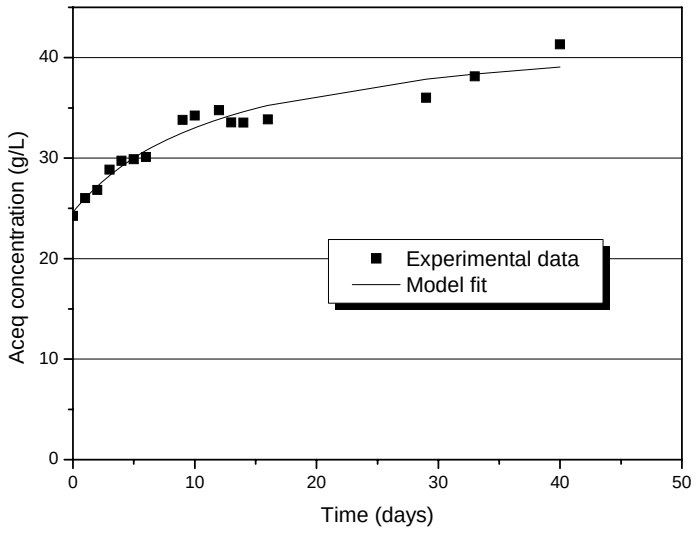


Figure 8-37. Aceq concentration for lime-treated bagasse/chicken manure batch fermentor at 100 g substrate + acids (b)/L liquid with ammonium bicarbonate.

The reaction rate and specific reaction rate for batch fermentations were calculated by using Equations 7-15 and 7-16. Conversion was calculated with the experimental acetic acid equivalents using Equation 7-17. Parameters *e*, *f*, *g*, and *h* present in the predicted rate equation (Equation 8-2) were calculated by nonlinear regression (*Systat Sigmaplot 10.0*). Figure 8-38 compares the predicted specific rate with the experimental specific rate. The specific rate equation for the 80 wt% hot-lime-water-treated bagasse/20 wt% chicken manure fermentation with ammonium bicarbonate buffer follows:

$$\hat{r}_{\text{pred}} = \frac{1.68(1-x)^{3.68}}{1+2.25(\phi \cdot \text{Aceq})^{0.926}} \tag{8-2}$$

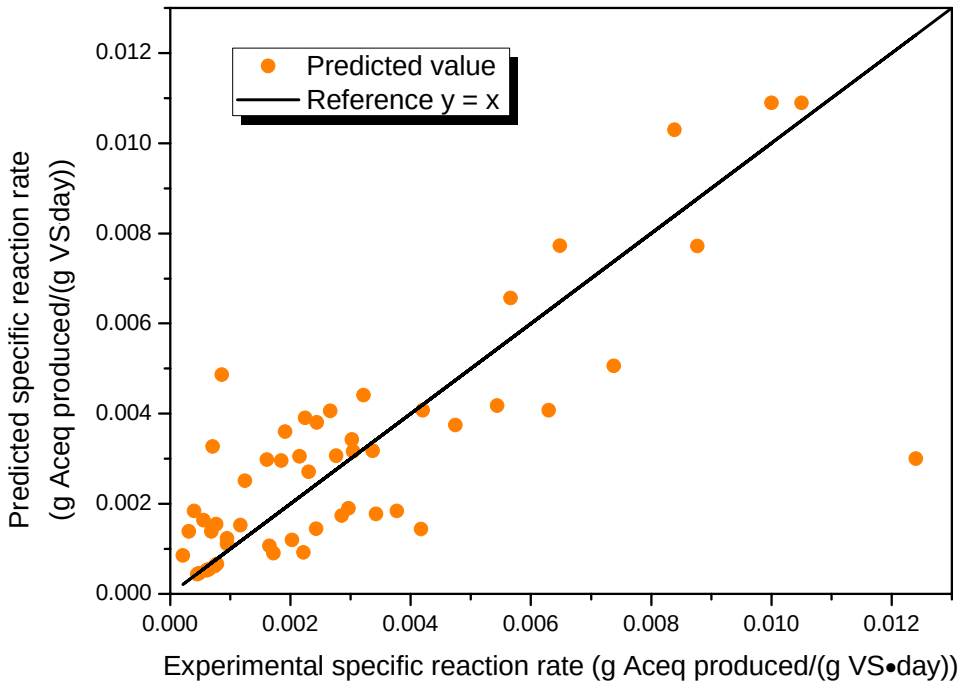


Figure 8-38. The experimental value and the CPDM prediction value for the specific reaction rate in five batch hot-lime-water-treated bagasse/chicken manure fermentations with ammonium bicarbonate.

Table 8-9. Parameter constant values in CPDM for hot-lime-water-treated bagasse/chicken manure fermentation system with ammonium bicarbonate.

Parameter constant	Train MA/MB	Train MC	Train MD/ME/MG
Holdup (g liquid/g VS cake)	4.41	4.44	4.49
Moisture (g liquid/g solid feed)	0.03		
Selectivity (g Aceq/g VS digested)	0.57	0.5	0.5
F1–F4 solid concentration (g VS/L)	108.7	88.1	130
F1–F4 liquid volume (L)	0.193	0.237	0.275
ϕ (g total acid/g Aceq)	0.89		
e (g Aceq/((g VS·d))	1.68		
f (dimensionless)	3.68		
g (L/g total acid)$^{1/h}$	2.25		
h (dimensionless)	0.926		

Table 8-9 lists the system-specific variables required by CPDM methods. Table 8-10 compares the experimental total carboxylic acid concentration and conversion to the CPDM prediction. As shown in Table 8-10, the total carboxylic acid concentrations from experiments agreed well with the CPDM predicted values, with an average absolute error of 9.06%. Substrate conversions for experimental and predicted conditions were very close, with an average absolute error of 14.17%.

Train MF is loaded with the highest VSLR of 131.35 g/(L·day). The fresh solid fed to F1 almost consumed all of free liquid in Fermentor F1. The centrifuged liquid on top of the wet cake in Fermentor F1 was detected very small and even zero. The CPDM program cannot run under such VSLR and LRT conditions. Therefore, Train MF is not compared in Table 8-10.

Table 8-10. Comparison of experimental and predicted carboxylic acid concentration for hot-lime-water-treated bagasse/chicken manure fermentations with ammonium bicarbonate.

	Train MA	**Train MB**	**Train MC**	**Train MD**	**Train ME**	**Train MG**	**Average** ** **(%)**
Experimental carboxylic acid concentration (g/L)	14.57	24.40	17.06	31.34	36.43	56.14	
Predicted (CPDM) carboxylic acid concentration (g/L)	17.04	26.11	16.32	33.53	41.29	52.93	
Error* (%)	16.95	7.01	-4.34	6.99	13.34	-5.72	9.06
Experimental conversion	0.67	0.62	0.66	0.76	0.66	0.44	
Predicted (CPDM) conversion	0.85	0.67	0.78	0.68	0.57	0.48	
Error* (%)	26.57	8.06	17.42	-11.05	-13.03	8.86	14.17

* Error (%) = ((Predicted value – Experimental value)/Experimental value) × 100

** Average errors are based on absolute value.

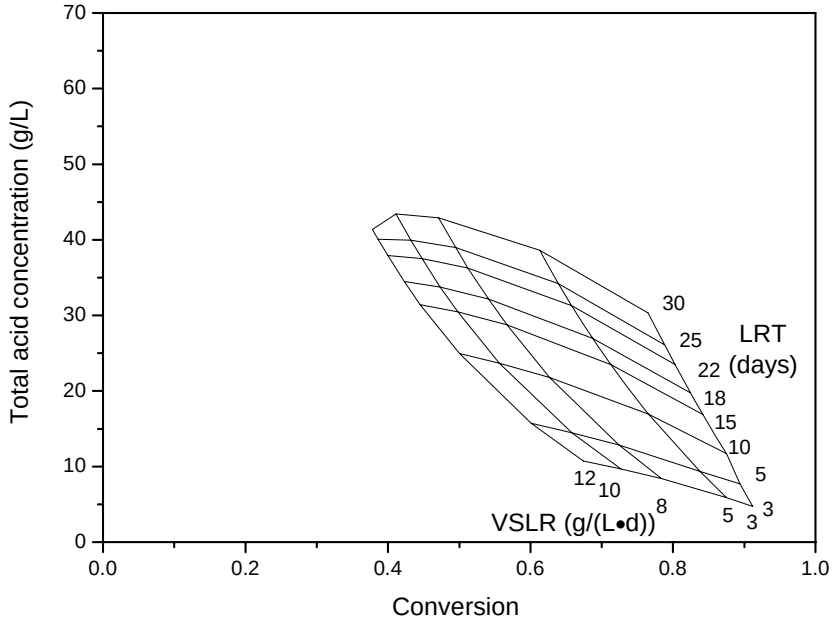


Figure 8-39. The CPDM "map" for 80 wt% hot-lime-water-treated bagasse/20 wt% chicken manure countercurrent fermentation with ammonium bicarbonate buffer (130 g VS/L liquid).

Figure 8-39 shows the CPDM "map" for hot-lime-water-treated bagasse / chicken manure countercurrent fermentation with ammonium bicarbonate at a fermentation solid concentration of 130 g VS/(L of liquid). The "map" predicts a total acid concentration of 43.42 g/L at LRT of 30 day, VSLR of 10 g/(L·d) and a conversion of 41.1%. At a VSLR of 3 g/(L·d) and LRT of 3 day, a total acid concentration of 3.721 g/L could be obtained at 90.2% conversion. A relatively high acid concentration (> 30 g/L) and high conversion (>75%) could be obtained at VSLR of 3 g/(L·d) and LRT of 30 day.

8.5 Summarized comparison of ammonium bicarbonate and calcium carbonate

The pH stability is different in the calcium carbonate buffered fermentations and ammonium bicarbonate buffered fermentations. Calcium carbonate is more stable at controlling pH. A typical pH in calcium carbonate buffered fermentation is 6.07±0.26, whereas the pH is more variable in ammonium bicarbonate buffered fermentations (e.g. 6.87±0.35 in Train ME). More pH control may be required in the pilot-scale fermentor for ammonium bicarbonate buffered fermentations. Automatic pH control is recommended for the industrial fermentor.

Higher substrate concentrations would be allowed if the process is operated on a large scale (Holtzapple et al., 1999). A higher VS concentration should result in higher total carboxylic acid concentrations. CPDM method was used to simulate this industrial application with a high solid concentration of 300 g VS/L.

Figure 8-40 predicts the calcium carbonate buffered fermentation behavior, whereas Figure 8-41 presents the simulated industrial fermentations with ammonium bicarbonate. As illustrated in the CPDM "map" of Figure 8-42, total acid concentrations as high as 30.47 g/L can be reached at LRT of 30 days, and VSLR of 10 g/(L·d) for calcium carbonate system. Also, conversions as high as 94.6% can be achieved at LRT of 2 days and VSLR of 2 g/(L·d). Both high conversions (> 60 %) and high product concentrations (> 25 g/L) can be achieved at LRT of 30 days and VSLR 5 g/(L·d).

Figure 8-41 shows fermentation behavior with ammonium bicarbonate on a large scale. As illustrated in the CPDM "map," total acid concentrations as high as 61.3 g/L can be reached at LRT of 30 days, and VSLR of 10 g/(L·d). Also, conversions as high as 93.0% can be achieved at LRT of 2 days and VSLR of 3 g/(L·d). Both high conversions (~ 75%) and high product concentrations (~ 50 g/L) can be achieved at LRT of 30 days and VSLR 5 g/(L·d).

In conclusion, ammonium bicarbonate is a better buffer than calcium carbonate in long-term countercurrent fermentations.

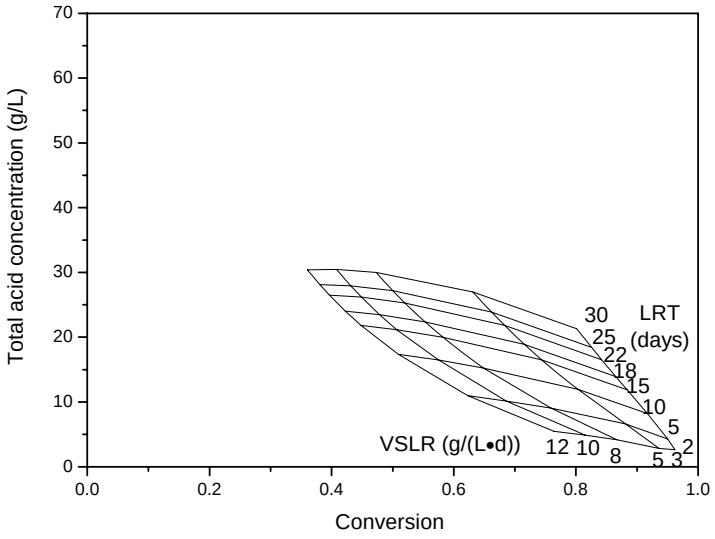


Figure 8-40. The CPDM "map" for 80 wt% hot-lime-water-treated bagasse/20 wt% chicken manure countercurrent fermentation with calcium carbonate buffer (300 g VS/L liquid).

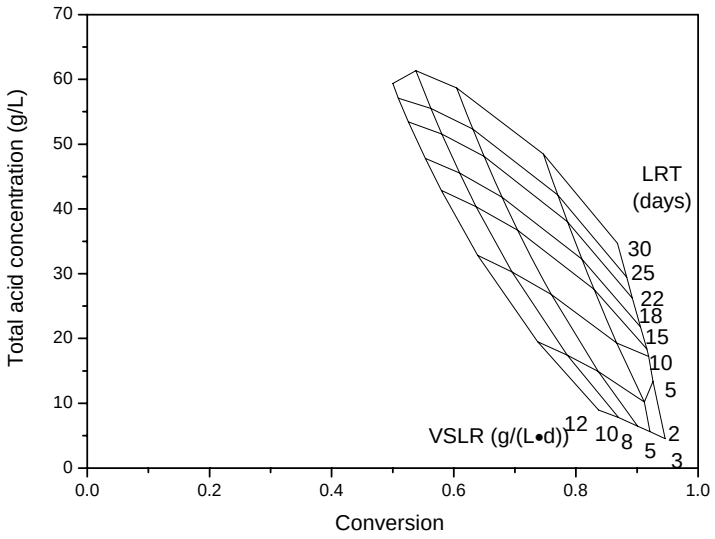


Figure 8-41. The CPDM "map" for 80 wt% hot-lime-water-treated bagasse/20 wt% chicken manure countercurrent fermentation with ammonium bicarbonate buffer (300 g VS/L liquid).

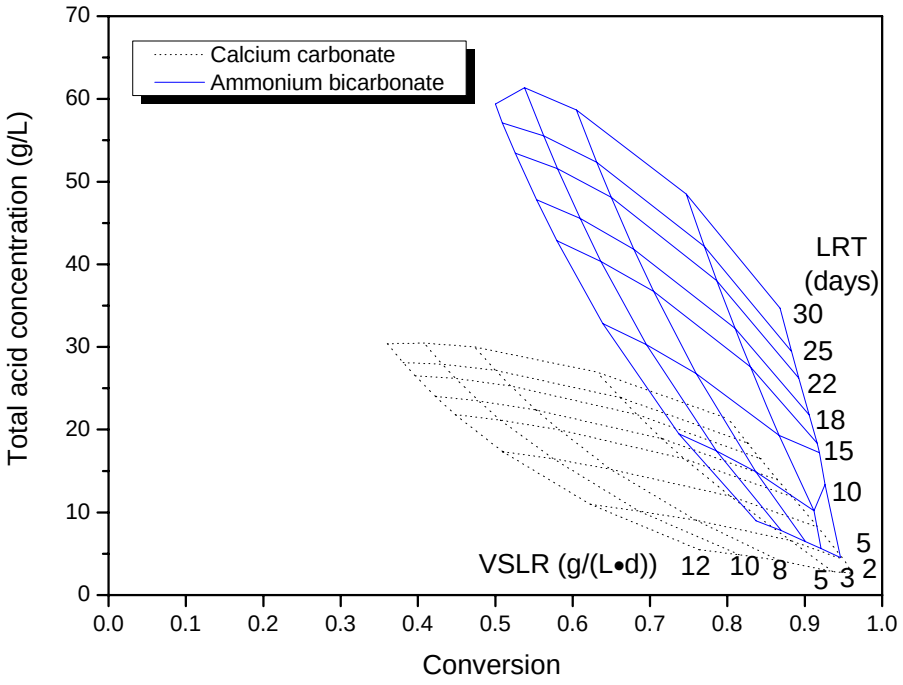


Figure 8-42. Comparison of CPDM "map" for 80 wt% hot-lime-water-treated bagasse/20 wt% chicken manure countercurrent fermentations (300 g VS/L liquid). Ammonium bicarbonate and calcium carbonate were used.

8.6 Conclusions

The following conclusions can be made based on the study in this chapter:

1) The long-term countercurrent fermentation shows that anaerobic microorganisms from the marine source can adapt to ammonium bicarbonate. Stable acid concentrations were achieved over 330 days fermentation time.

2) For hot-lime-water-treated bagasse fermentations buffered by ammonium bicarbonate at a VS concentration of 130 g/L, a total acid concentration of 43.42 g/L was achieved at LRT of 30 day, VSLR of 10 g/(L·d), and a conversion of 41.1%. At a VSLR of 3 g/(L·d) and LRT of 3 day, a total acid concentration of 3.72 g/L could be obtained at 90.2% conversion.

3) For hot-lime-water-treated bagasse fermentations buffered by calcium carbonate at a VS concentration of 130 g/L, a total acid concentration of 20.53 g/L was achieved at LRT of 30 day, VSLR of 8 g/(L·d), and a conversion of 34.0%. At a VSLR of 2.5 g/(L·d) and LRT of 3 day, a total acid concentration of 2.47 g/L could be obtained at 92.9% conversion.

4) Ammonium bicarbonate is a better buffer than calcium carbonate. Higher acid concentrations were achieved in ammonium bicarbonate fermentation.

5) The CPDM method is a powerful tool to predict product concentration and conversion based on batch fermentation data. The experimental acid concentration and conversion agree well with the CPDM prediction (average absolute error < 15%) in both countercurrent fermentations using ammonium bicarbonate and using calcium carbonate buffers.

CHAPTER IX
LONG-TERM EFFECTS OF PRETREATMENT METHODS ON AMMONIUM BICARBONATE BUFFERED FERMENTATIONS

The objectives of this chapter follow:

a) To evaluate different pretreatment methods on long-term bagasse fermentations using a mixed culture of anaerobic marine microorganisms.

b) To apply the CPDM method to different treated bagasse fermentations, and compare both acid concentration and conversion with experimental values.

c) To predict the optimized acid concentration and conversion in industrial long-term fermentations for different treated bagasse using the CPDM method.

d) To recommend industrial biomass conversion using combinations of the studied pretreatments and fermentations.

9.1 Introduction

Pretreatment is an important step for lignocellulosic biomass conversion. It is required to disrupt the hemicellulose/lignin sheath that surrounds the cellulose, and therefore makes cellulose more accessible to enzymes that convert carbohydrate polymers into fermentable sugars (see Figure 9-1). Pretreatment has been regarded as one of the most expensive processing steps in lignocellulosic biomass-to-fermentable sugars conversion with costs as high as 30¢/gallon ethanol produced (Mosier et al. 2005; Wyman et al. 2005).

Pretreatment methods can be physical, or biological, or chemical. Some methods incorporate both physical and chemical effects. Physical pretreatments, including high temperature, freeze/thaw cycles, and radiation, are aimed at size reduction and mechanical decrystallization. Most of these methods are limited in their effectiveness and are often expensive. Biological pretreatments, where natural organisms are allowed to grow on the biomass, result in cellulose and lignin degradation but are not very effective and require long treatment times. Therefore, chemically based approaches have gained the most significant attention.

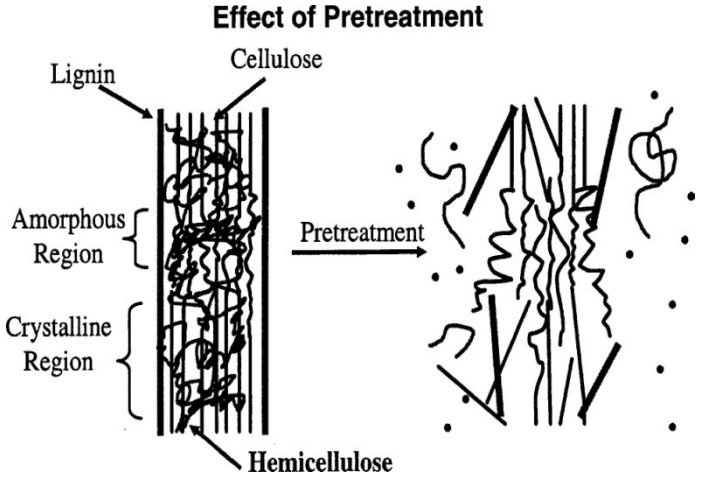


Figure 9-1. Schematic of goals of pretreatment on lignocellulosic biomass (Hsu et al. 1980).

Various chemical pretreatment methods have been proposed. Dilute acid and alkali pretreatments are the focus of current research interest. Pretreatments using dilute acid (e.g., sulfuric acid) and steam or pressurized hot water achieve high yields of soluble sugars from the hemicellulose fraction of biomass. The hot-wash process, a variation of the dilute acid pretreatment, involves high-temperature separation and washing of the pretreated solids, which is thought to prevent re-precipitation of lignin and/or xylan that may have been solubilized under pretreatment conditions. Ammonia fiber explosion (AFEX) disrupts lignocellulose and reduces the cellulase requirement but removes neither hemicellulose nor lignin. Alkali pretreatment is so far relatively suitable for lignocellulosic biomass, because it successfully removes lignin and can be performed at lower temperatures and pressures compared to other pretreatments, such as dilute acid and steam explosion (Mosier et al. 2005). Alkali pretreatment are generally more effective at solubilizing a greater fraction of lignin while leaving behind much of the hemicellulose in an insoluble, polymeric form.

Alkali pretreatments mainly use lime and ammonia. Lime is widely used in the traditional MixAlco process (Section 1.2). Other than lime, ammonia is also an effective reagent due to its ability to swell lignocellulosic biomass, its high selectivity for reactions with lignin over carbohydrates, and its high volatility rendering it easy to recycle and reuse (Iyer et al. 1996; Kim et al. 2003). Ammonia recycled percolation (ARP) pretreatment uses aqueous ammonia in a flow-through reactor packed with biomass at temperatures from 160^{o}C to 180^{o}C (Iyer et al. 1996; Yoon et al. 1995). Another successful alternative method to ARP simply consists of soaking biomass in aqueous ammonia for 24 hours at 65^{o}C (Kim and Lee 2005b).

In summary, none of the current pretreatment technologies (e.g., dilute acid, hot water, lime, and ammonia) is entirely mature. This chapter compares effects of biomass pretreatments on long-term ammonium bicarbonate buffered fermentations. The

objective of this chapter is to seek suitable biomass treatment methods for the desired ammonium bicarbonate buffered fermentations.

9.2 Materials and methods

Two different treatment methods were selected in this study. They were air-lime pretreatment (i.e., lime treatment at 55°C with a treatment time of 2 months) and aqueous ammonia pretreatment. Both experimental results and CPDM prediction of carboxylic acid concentration in countercurrent fermentations at various volatile solid loading rates (VSLR) and liquid residence time (LRT) are presented in this chapter.

The thermophilic fermentations used in this chapter are four-stage countercurrent fermentations. Treated sugarcane bagasse (80%) and chicken manure (20%) were used as substrates in the rotary fermentors. All fermentation trains were inoculated with a mixed culture of anaerobic microorganisms from marine source (sediments from different locations in Galveston Island, TX). All fermentations were operated at 55°C (thermophilic condition). Four fermentations were started as batch fermentations with treated bagasse (80%) and chicken manure (20%), dry nutrient mixture, and deoxygenated water. Ammonium bicarbonate was the only pH buffer used in this chapter. The single-centrifuge procedure, where liquids are transferred in a single step, was used in all countercurrent fermentations. The transfer of liquid and solids was operated at 2-day intervals for all fermentation trains in this chapter. After the steady state is achieved (±5 g/L total acid concentration), fermentation data were collected for at least 10 transfers to determine acid productivity, carboxylic acid concentration, yield, selectivity, conversion, biotic carbon dioxide, and methane production.

Five different batch fermentations were established to obtain the CPDM parameters for the different fermentation systems. The detailed batch fermentation procedures for CPDM methods are described in Chapter VII.

9.3 Countercurrent fermentations using hot-lime-water treatment

Extensive studies were performed for countercurrent fermentations coupled with hot-lime-water treatment (2 hours and 105°C). More details can be referred to Section 8.2 in Chapter VIII.

9.4 Countercurrent fermentations using ammonia-treated bagasse

In this section, ammonia treatment (Appendix C) was utilized to enhance biomass digestibility. Ammonium bicarbonate is the only pH buffer used in this section to control the desired pH 7.0 (6.97–7.03). The transfer of liquids and solids for all trains in this section were operated at a two-day interval. The preset constant wet weight of solid cake was 300 g. A series of six fermentation trains were used to examine the ammonia-treated bagasse: Trains MH, MK, ML, NH, NK, and NL.

9.4.1 Train MH

Four batch fermentations were initiated by adding ammonia-treated bagasse (32 g), chicken manure (8 g), ammonium bicarbonate (2 g), nutrient mixture (0.2 g), marine inocula (40 mL), anaerobic water (360 mL), and 120 μL iodoform solution (20 g/L of iodoform dissolved in ethanol). The marine inocula were taken from a previous batch of ammonia-treated bagasse and chicken manure fermentations with ammonium bicarbonate (Chapter IV). On each transfer with Train MH, ammonia-treated bagasse (6.4 g), chicken manure (1.6 g), nutrients (0.2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g) and iodoform (60 μL) were added to F2, F3, and F4. Ammonium bicarbonate was added to control the pH in fermentation broth around 7.0 (6.97–7.03). The transfer of solids and liquids was performed as shown in Chapter VII. Fresh anaerobic water (100 mL) was added to F4 on each transfer. The total acid concentration profile and acetate content profile are shown in Figures 9-2 and 9-3.

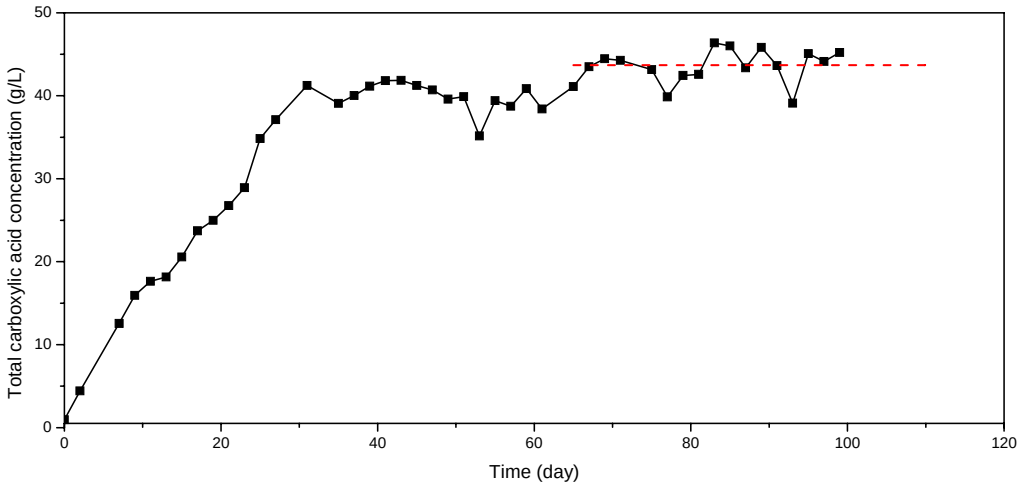


Figure 9-2. Total acid concentration ammonia-treated bagasse Fermentation Train MH (ammonium bicarbonate, fresh solid 8 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (43.69 g/L).

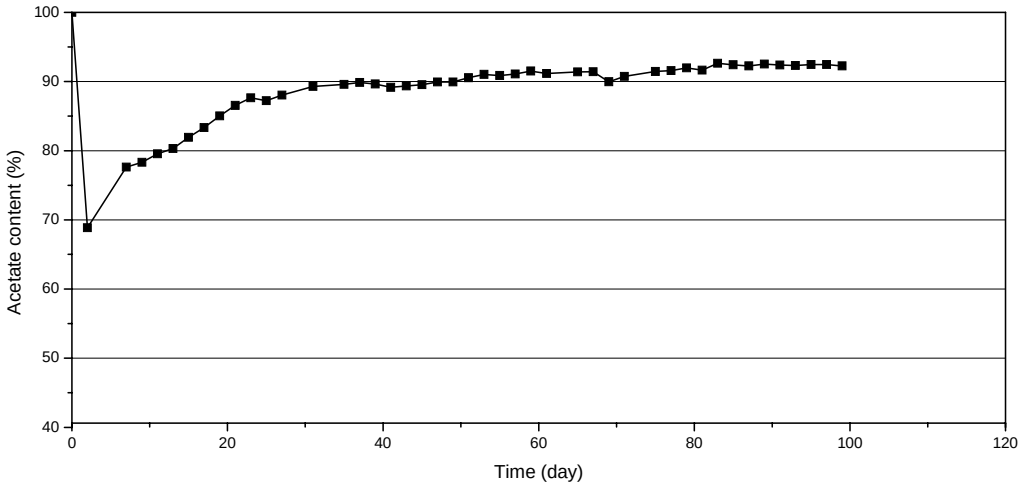


Figure 9-3. Acetate content for ammonia-treated bagasse Fermentation Train MH (ammonium bicarbonate, fresh solid 8 g, fresh liquid 100 mL, and constant cake weight 300 g).

9.4.2 Train MK

Four batch fermentations were initiated by adding 32 g of ammonia-treated bagasse, chicken manure (8 g), ammonium bicarbonate (2 g), nutrient mixture (0.2 g), marine inocula (40 mL), anaerobic water (360 mL), and 120 μL iodoform solution (20 g/L of iodoform dissolved in ethanol). The marine inocula were taken from a previous batch of ammonia-treated bagasse and chicken manure fermentations with ammonium bicarbonate buffer (Chapter IV). On each transfer with Train MK, ammonia-treated bagasse (12.8 g), chicken manure (3.2 g), nutrients (0.2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g) and iodoform (60 μL) were added to F2, F3, and F4. ammonium bicarbonate was added to control the pH in the fermentation broth around 7.0 (6.97–7.03). The transfer of solids and liquids was performed as shown in Chapter VII. Fresh anaerobic water (100 mL) was added to F4 on each transfer. The total acid concentration profile and acetate content profile are shown in Figures 9-4 and 9-5.

9.4.3 Train ML

Four batch fermentations were initiated by adding ammonia-treated bagasse (32 g), chicken manure (8 g), ammonium bicarbonate (2 g), nutrient mixture (0.2 g), marine inocula (40 mL), anaerobic water (360 mL), and 120 μL iodoform solution (20 g/L of iodoform dissolved in ethanol). The marine inocula were taken from a previous batch of ammonia-treated bagasse and chicken manure fermentations with ammonium bicarbonate buffer (Chapter IV). On each transfer with Train ML, ammonia-treated bagasse (9.6 g), chicken manure (2.4 g), nutrients (0.2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g) and iodoform (60 μL) were added to F2, F3, and F4. Ammonium bicarbonate was added to control the pH in the fermentation broth around 7.0 (6.97–7.03). The transfer of solids and liquids was performed as shown in Chapter VII. Fresh anaerobic water (100 mL) was added to F4 on each transfer. The total acid concentration profile and acetate content profile are shown in Figures 9-6 and 9-7.

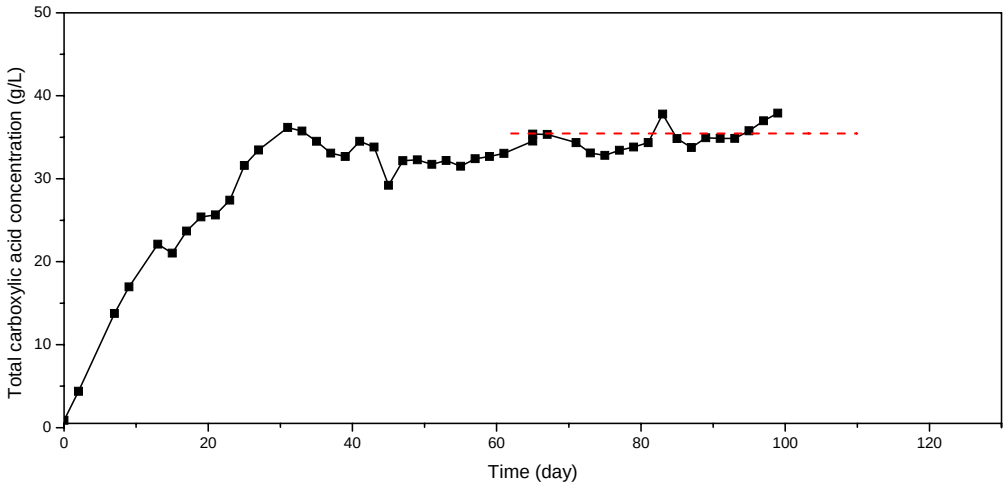


Figure 9-4. Total acid concentration ammonia-treated bagasse Fermentation Train MK (ammonium bicarbonate, fresh solid 16 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (35.44 g/L).

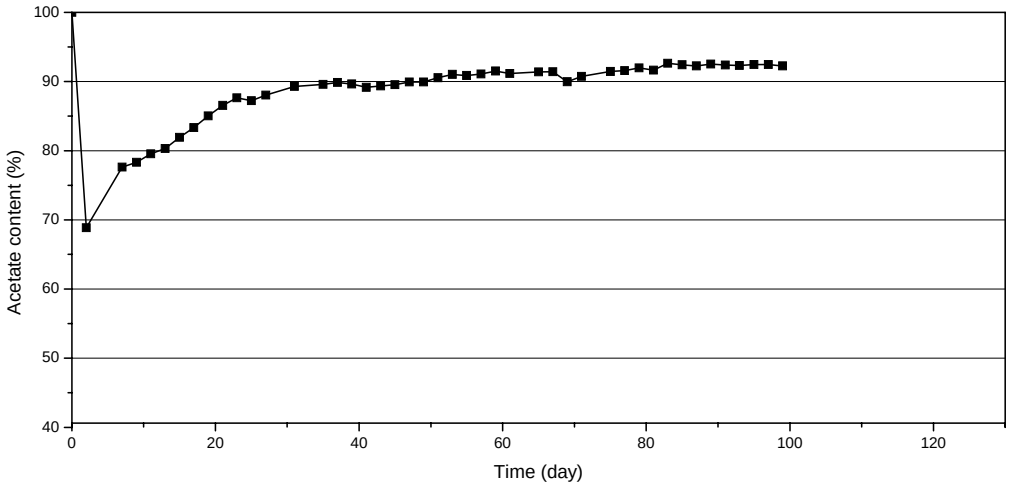


Figure 9-5. Acetate content for ammonia-treated bagasse Fermentation Train MK (ammonium bicarbonate, fresh solid 16 g, fresh liquid 100 mL, and constant cake weight 300 g).

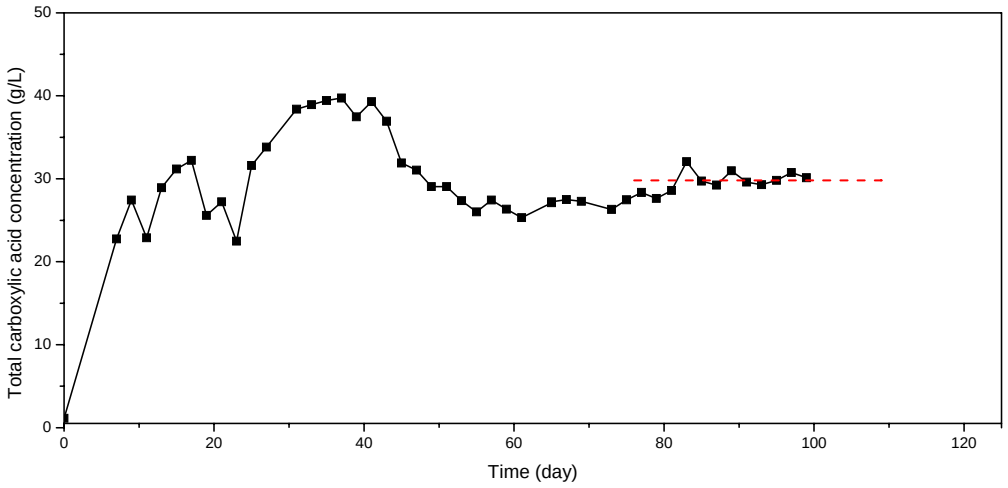


Figure 9-6. Total acid concentration ammonia-treated bagasse Fermentation Train ML (ammonium bicarbonate, fresh solid 12 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (29.79 g/L).

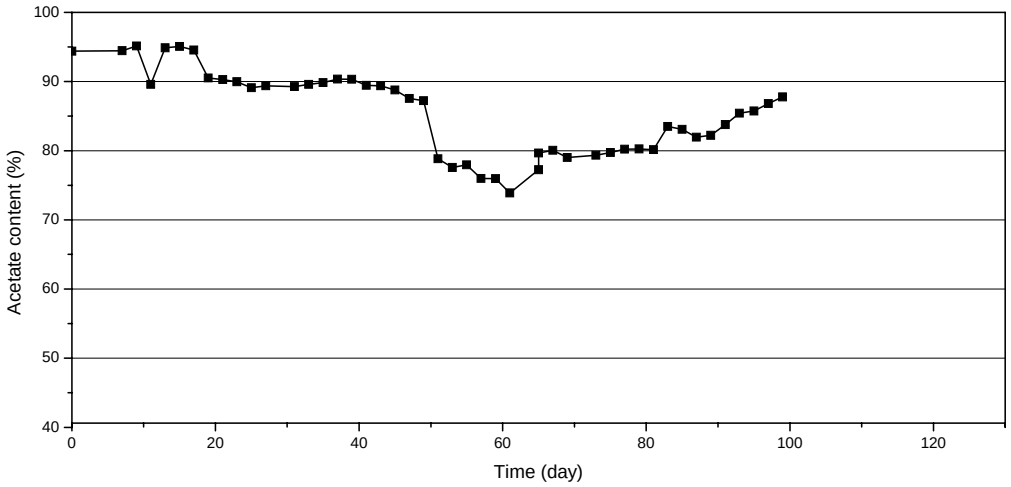


Figure 9-7. Acetate content for ammonia-treated bagasse Fermentation Train ML (ammonium bicarbonate, fresh solid 12 g, fresh liquid 100 mL, and constant cake weight 300 g).

9.4.4 Train NH

Train NH was a continuation of Train MH, but operated with a different solid feed ratio (14.4 g fresh biomass to F1). Train NH did not redistribute the solid and liquid of Train MH. There was no batch stage for Train NH. On each transfer with Train NH, ammonia-treated bagasse (11.52 g), chicken manure (2.88 g), nutrients (0.2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g) and iodoform (60 μL) were added to F2, F3, and F4. Ammonium bicarbonate was added to control the pH in the fermentation broth around 7.0 (6.97–7.03). The transfer of solids and liquids was performed as shown in Chapter VII. Fresh anaerobic water (100 mL) was added to F4 on each transfer. The total acid concentration profile and acetate content profile are shown in Figures 9-8 and 9-9.

9.4.5 Train NK

Train NK was a continuation of Train MK, but operated with a different solid feed ratio (10.8 g fresh biomass to F1). Train NK did not redistribute the solid and liquid of Train MK. There was no batch stage for Train NK. On each transfer with Train NK, ammonia-treated bagasse (8.64 g), chicken manure (2.16 g), nutrients (0.2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g) and iodoform (60 μL) were added to F2, F3, and F4. Ammonium bicarbonate was added to control the pH in the fermentation broth around 7.0 (6.97–7.03). The transfer of solids and liquids was performed as shown in Chapter VII. Fresh anaerobic water (100 mL) was added to F4 on each transfer. The total acid concentration profile and acetate content profile are shown in Figures 9-10 and 9-11.

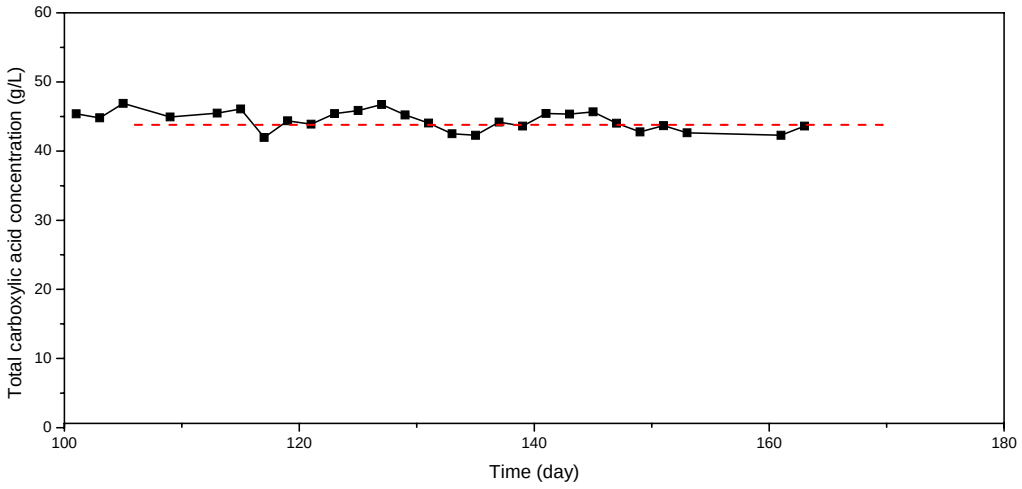


Figure 9-8. Total acid concentration for ammonia-treated bagasse Fermentation Train NH (ammonium bicarbonate, fresh solid 14.4 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (43.79 g/L).

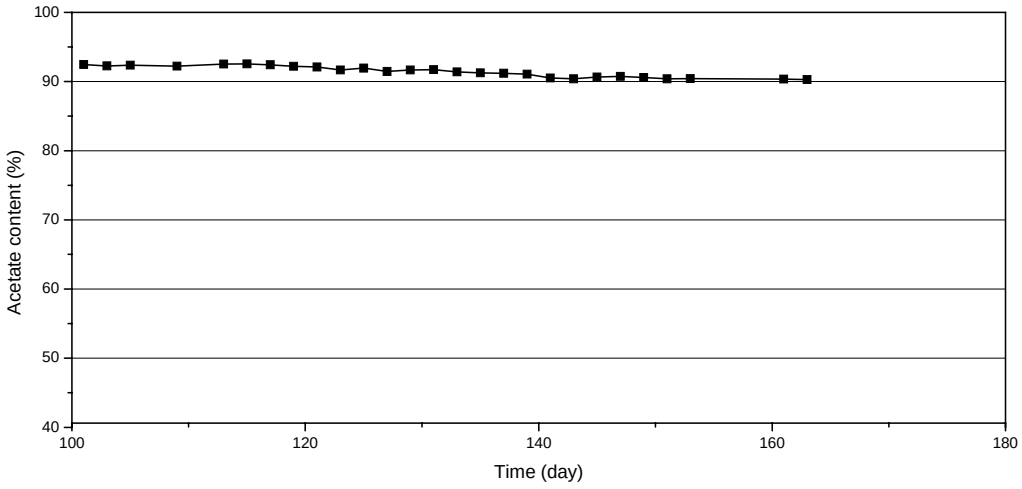


Figure 9-9. Acetate content for ammonia-treated bagasse Fermentation Train NH (ammonium bicarbonate, fresh solid 14.4 g, fresh liquid 100 mL, and constant cake weight 300 g).

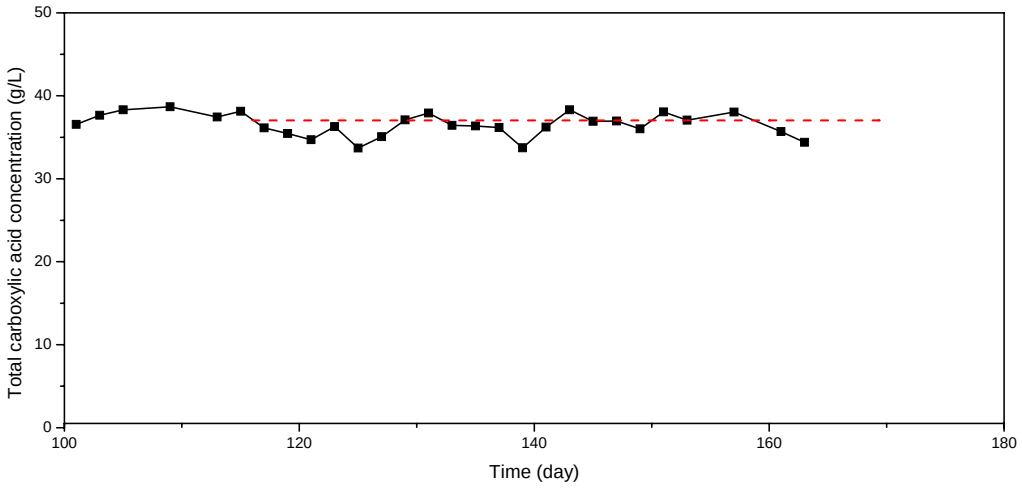


Figure 9-10. Total acid concentration for ammonia-treated bagasse Fermentation Train NK (ammonium bicarbonate, fresh solid 10.8 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (37.03 g/L).

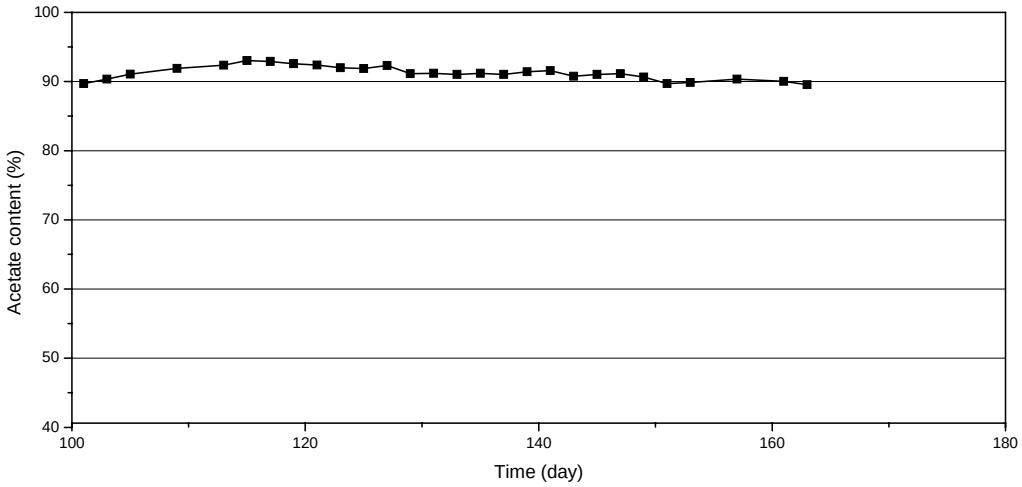


Figure 9-11. Acetate content for ammonia-treated bagasse Fermentation Train NK (ammonium bicarbonate, fresh solid 10.8 g, fresh liquid 100 mL, and constant cake weight 300 g).

9.4.6 **Train NL**

Train NL was a continuation of Train ML, but operated with a different solid feed ratio (7.2 g fresh biomass to F1). Train NL did not redistribute the solid and liquid of Train ML. There was no batch stage for Train NL. On each transfer with Train NL, ammonia-treated bagasse (5.76 g), chicken manure (1.44 g), nutrients (0.2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g) and iodoform (60 μL) were added to F2, F3, and F4. Ammonium bicarbonate was added to control the pH in the fermentation broth around 7.0 (6.97–7.03). The transfer of solids and liquids was performed as shown in Chapter VII. Fresh anaerobic water (100 mL) was added to F4 on each transfer. The total acid concentration profile and acetate content profile are shown in Figures 9-12 and 9-13.

9.4.7 Summary of ammonia-treated bagasse fermentations

Table 9-1 summarizes the operating conditions for Trains MH, MK, ML, NH, NK, and NL, whereas Table 9-2 shows the fermentation results for the countercurrent fermentations using ammonia-treated bagasse. Figures 9-14 and 9-15 list the mass balance closures for these fermentations.

The highest acid productivity of 1.16 g/(L·day) occurred at a concentration of 35.44 g/L in Fermentation Train MK (LRT = 30.6 day and VSLR = 4.42 g/(L·day)). Fermentation Train NL (LRT = 29.9 day and VSLR = 2.74 g/(L·day)) with a concentration of 27.64 g/L had the highest conversion (0.65 g VS digested/g VS fed) and yield (0.34 g total acids/g VS fed). Fermentation Train NL had the highest conversion because it had the lowest VSLR, which made more complete use of the biomass. The highest selectivity of 0.75 g total acids/g VS digested was in fermentation train MK (LRT = 30.63 d and VSLR = 4.42 g/(L·day))

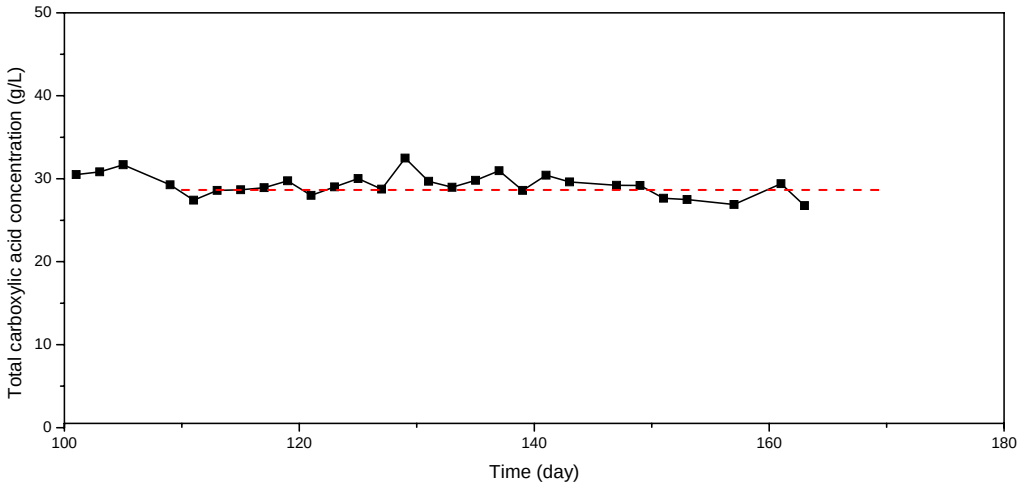


Figure 9-12. Total acid concentration for ammonia-treated bagasse Fermentation Train NL (ammonium bicarbonate, fresh solid 7.2 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (27.64 g/L).

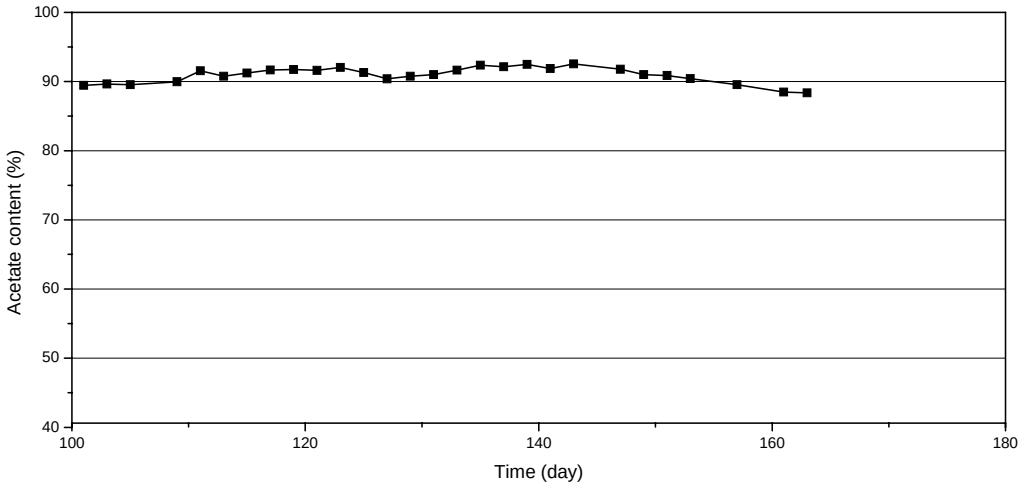


Figure 9-13. Acetate content for ammonia-treated bagasse Fermentation Train NL (ammonium bicarbonate, fresh solid 7.2 g, fresh liquid 100 mL, and constant cake weight 300 g).

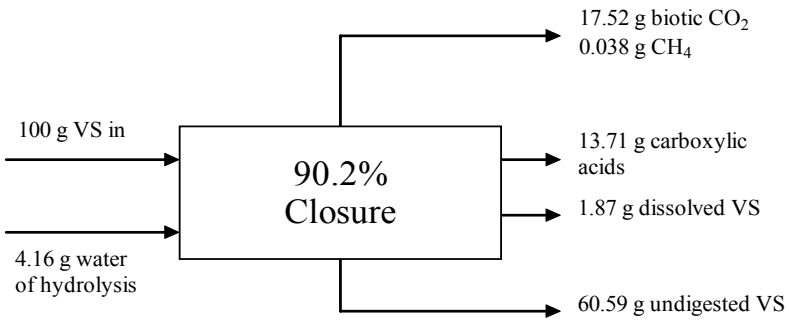
Table 9-1. Operating parameters for ammonia-treated bagasse countercurrent fermentation**.**

Fermentation Trains	MH	MK	ML	NH	NL	NK
LRT (day)	55.48	30.63	26.22	45.18	29.94	32.85
VSLR (g VS/L liquid in all fermentors·day)	5.74	4.42	3.07	5.30	2.74	4.19
VS feed at each transfer (g VS)	14.02	10.51	7.01	12.61	6.31	9.46
Solid feed at each transfer (g)	16.00	12.00	8.00	14.40	7.20	10.80
Treated bagasse (g)	12.80	9.60	6.40	11.52	5.76	8.64
Chicken manure (g)	3.20	2.40	1.60	2.88	1.44	2.16
Liquid fed to F4 at each transfer (L)	0.10	0.10	0.10	0.10	0.10	0.10
VS/liquid feed ratio (g VS/g liquid)	0.14	0.11	0.07	0.13	0.06	0.09
Liquid volume in all four fermentors (L)	1.22	1.19	1.14	1.19	1.15	1.13
Temperature (ºC)	55					
Frequency of transfer	Every two days					
Centrifuge Procedure	Single					
F_1 Retained weight (wet g)	284	288	292	285.6	292.8	289.2
F_2–F_4 Retained weight (wet g)	300	300	300	300	300	300
Iodoform addition rate (mg iodoform added/L liquid fed to F4)	24	24	24	24	24	24
Nutrients addition rate (g dry nutrients added/L liquid fed to F4)	2.0	2.0	2.0	2.0	2.0	2.0
Urea addition rate (g urea added/L liquid feed to F4)	0.0	0.0	0.0	0.0	0.0	0.0

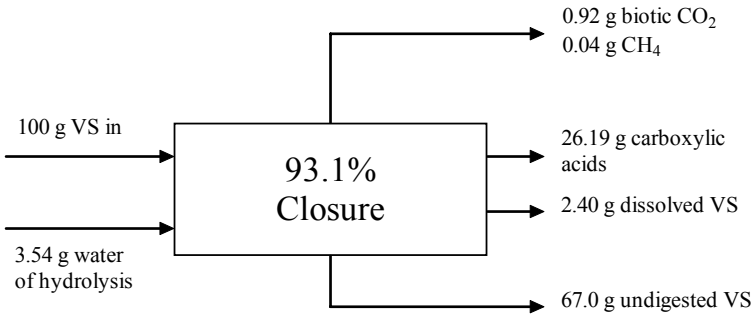
Table 9-2. Fermentation results for ammonia-treated bagasse countercurrent fermentation.

Fermentation Trains	MH	MK	ML	NH	NL	NK
Average pH in all fermentors	7.14±0.32	7.19±0.38	7.13±0.27	7.04±0.33	7.17±0.37	7.13±0.39
Total carboxylic acid concentration (g/L)	43.69±2.02	35.44±1.48	29.79±1.19	43.79±1.20	27.64±1.06	37.03±0.94
Acetic acid (wt%)	92.01±0.93	87.98±0.48	83.70±2.51	90.64±0.34	89.54±1.13	90.56±0.63
Propionic acid (wt%)	3.51±0.48	3.07±0.22	2.43±0.15	3.43±0.22	2.83±0.30	3.16±0.34
Butyric acid (wt%)	4.41±0.24	8.51±0.30	13.18±2.61	5.93±0.26	7.13±0.77	6.18±0.58
valeric acid (wt%)	0.16±0.14	0.45±0.04	0.70±0.06	0.00±0.00	0.50±0.09	0.10±0.15
Caproic acid (wt%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Heptanoic acid (wt%)	0.04±0.10	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Conversion (g VS digested/g VS fed)	0.41	0.35	0.53	0.40	0.65	0.41
Yield (g total acids/g VS fed)	0.14	0.26	0.37	0.18	0.34	0.14
Selectivity (g total acids/g VS digested)	0.34	0.75	0.69	0.45	0.52	0.34
Total carboxylic acid productivity (g total acids/ (L liquid·day))	0.79	1.16	1.14	0.97	0.92	0.79
Methane productivity (g CH_4/(L liquid·day))	0.0022	0.0018	0.0003	0.0008	0.0020	0.0004
Mass balance closure (g VS out/g VS in)	0.902	0.931	1.083	1.009	0.949	1.010

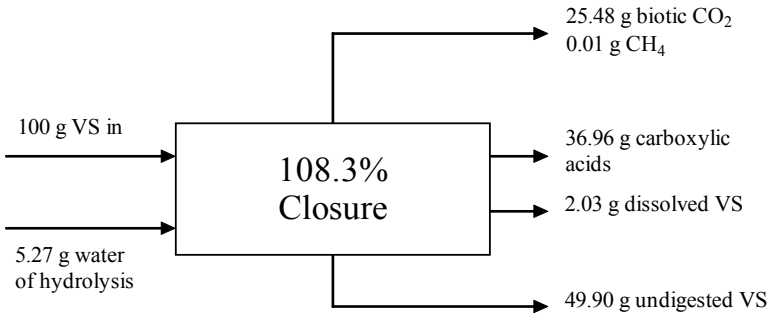
Note: All errors are ± 1 standard deviation.



(a) For Fermentation MH.

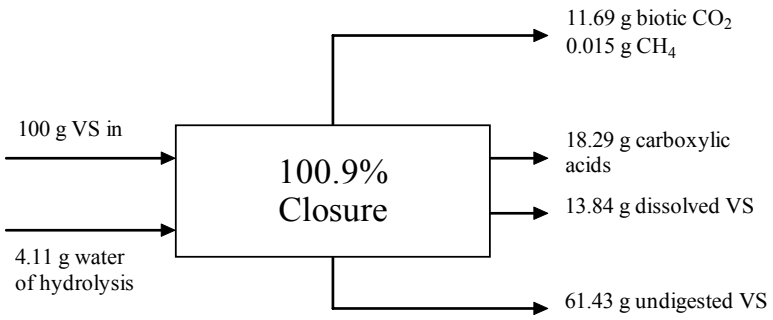


(b) For Fermentation MK.

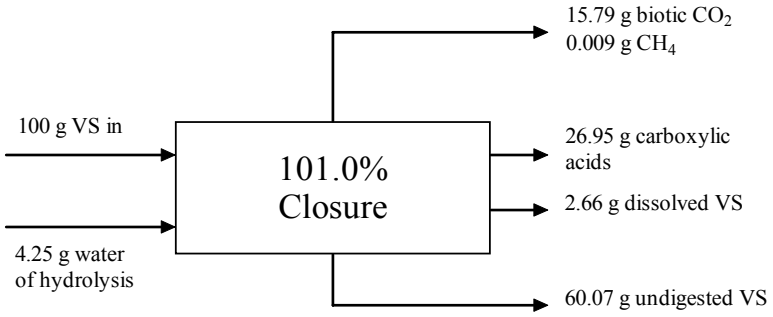


(c) For Fermentation ML.

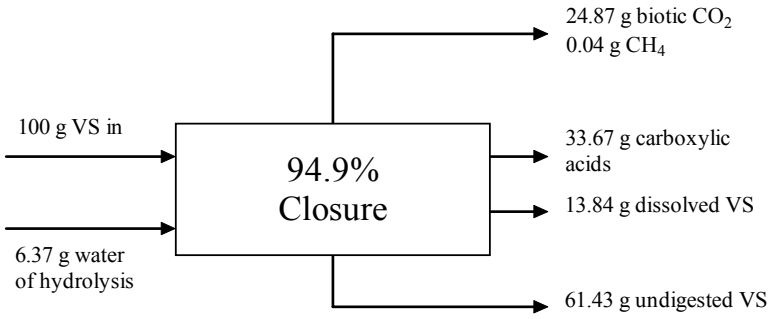
Figure 9-14. Mass balances for ammonia-treated bagasse Fermentations MH, MK, and ML.



(a) For Fermentation NH.



(b) For Fermentation NK.



(c) For Fermentation NL.

Figure 9-15. Mass balances for ammonia-treated bagasse Fermentations NH, NK, and NL.

9.5 Countercurrent fermentations using air-lime treated bagasse

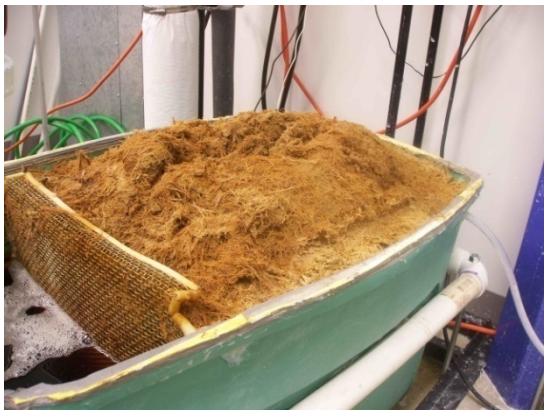
In this section, an improved lime-treatment (air-lime treatment) for sugarcane bagasse was utilized to enhance biomass digestibility. Raw sugarcane bagasse, water, and desired amount of lime (e.g., 0.3 g $Ca(OH)_2$/g dry biomass) were fully mixed and packed in the self-constructed long-term lime treatment system (Figure 9-16 a). A lime slurry container (Figure 9-16 b) was used to prevent lime in the pretreatment bed from being consumed by carbon dioxide from air feed. This specially treated air was continuously bubbled into the pretreatment system at a controlled speed (Appendix B). After 2 months of pretreatment, bagasse was harvested (Figure 9-16 d) and cooled inside a metal tray to room temperature. Once the biomass was cooled, CO_2 gas was bubbled into the biomass slurry to neutralize the excess lime. The resulting biomass was dried in the oven at 105^{o}C for 2 days. Dried air-lime treated bagasse was ready for long-term countercurrent fermentations.

Air-lime-treated bagasse (80 wt%) and chicken manure (20 wt%) were used as substrates in the rotary fermentors. All fermentation trains in this section were inoculated with marine inocula (sediments from different locations in Galveston Island, TX). All fermentations were operated at 55°C (i.e., thermophilic condition). Ammonium bicarbonate was the pH buffer used to maintain pH around 7.0. A series of three fermentation trains (Trains TA, TB, and TC) were used to examine the long-term fermentation performance of air-lime-treated bagasse.

(a) Overview of air-lime biomass treatment system.

(b) Lime slurry container.

(c) Biomass treatment "bed" to hold bagasse.



(d) Harvested bagasse after air-lime treatment with a treatment time of 2 months.

Figure 9-16. Photographies of air-lime biomass pretreatment system.

9.5.1 Train TA

Four batch fermentations were initiated by adding air-lime-treated bagasse (32 g), chicken manure (8 g), ammonium bicarbonate (2 g), nutrient mixture (0.2 g), marine inocula (40 mL), anaerobic water (360 mL), and 120 μL iodoform solution (20 g/L of iodoform dissolved in ethanol). The marine inocula were taken from a previous batch of air-lime-treated bagasse and chicken manure fermentations with ammonium bicarbonate buffer (Chapter V). On each transfer with Train TA, air-lime-treated bagasse (12.8 g), chicken manure (3.2 g), nutrients (0.2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g) and iodoform (60 μL) were added to F2, F3, and F4. Ammonium bicarbonate was added to control the pH in the fermentation broth around 7.0 (6.97–7.03). The transfer of solids and liquids was performed as shown in Chapter VII. The transfer of liquids and solids was operated at a two-day interval for Train TA. Fresh anaerobic water (100 mL) was added to F4 on each transfer. The total acid concentration profile and acetate content profile are shown in Figures 9-17 and 9-18.

9.5.2 Train TB

Four batch fermentations were initiated by adding air-lime-treated bagasse (32 g), chicken manure (8 g), ammonium bicarbonate (2 g), nutrient mixture (0.2 g), marine inocula (40 mL), anaerobic water (360 mL), and 120 μL iodoform solution (20 g/L of iodoform dissolved in ethanol). The marine inocula were taken from a previous batch of air-lime-treated bagasse and chicken manure fermentations with ammonium bicarbonate buffer (Chapter V). On each transfer with Train TB, air-lime-treated bagasse (9.6 g), chicken manure (2.4 g), nutrients (0.2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g) and iodoform (60 μL) were added to F2, F3, and F4. Ammonium bicarbonate was added to control the pH in the fermentation broth around 7.0 (6.97–7.03). The transfer of solids and liquids was performed as shown in Chapter VII. The transfer of liquids and solids was operated at a two-day interval for Train TB. Fresh anaerobic water (100 mL) was added to F4 on each transfer. The total acid concentration profile and acetate content profile are shown in Figures 9-19 and 9-20.

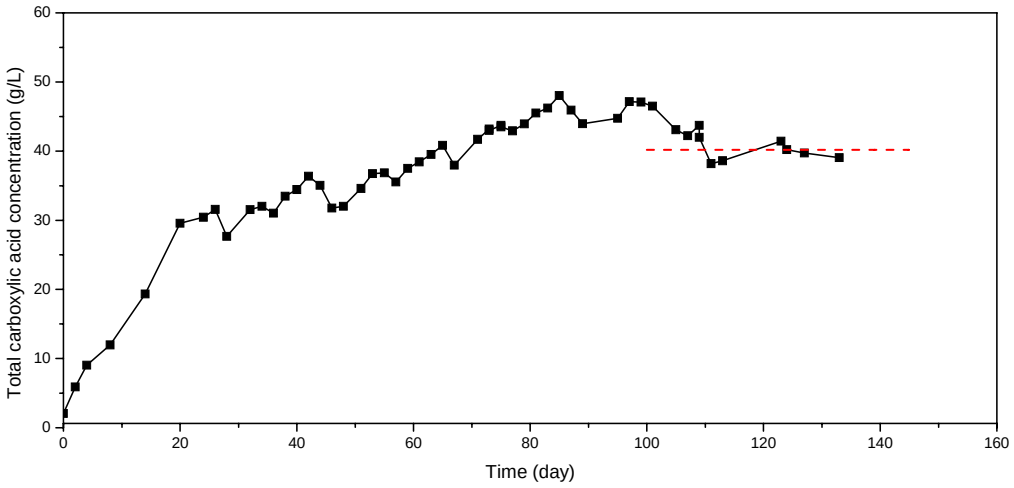


Figure 9-17. Total acid concentration for air-lime-treated bagasse Fermentation Train TA (ammonium bicarbonate, fresh solid 16 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (40.18 g/L).

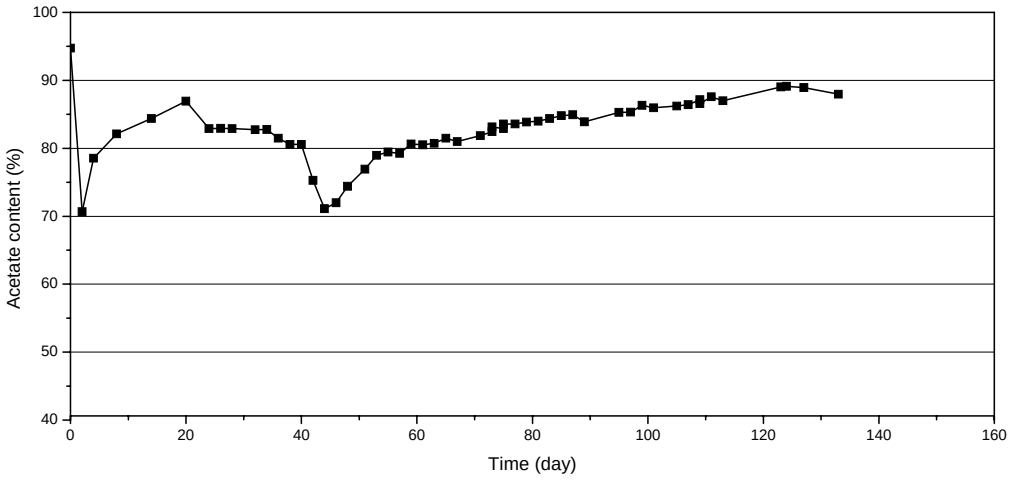


Figure 9-18. Acetate content for air-lime-treated bagasse Fermentation Train TA (ammonium bicarbonate, fresh solid 16 g, fresh liquid 100 mL, and constant cake weight 300 g).

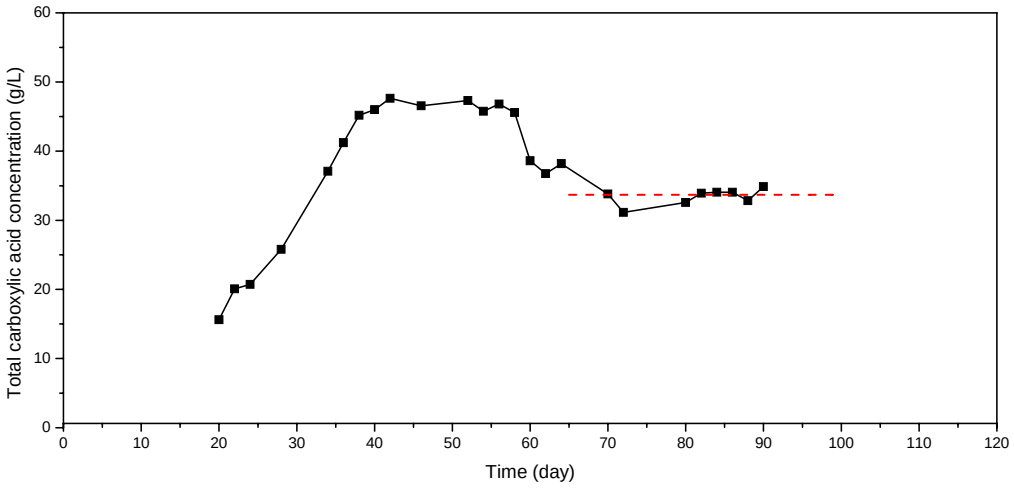


Figure 9-19. Total acid concentration for air-lime-treated bagasse Fermentation Train TB (ammonium bicarbonate, fresh solid 12 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (33.71 g/L).

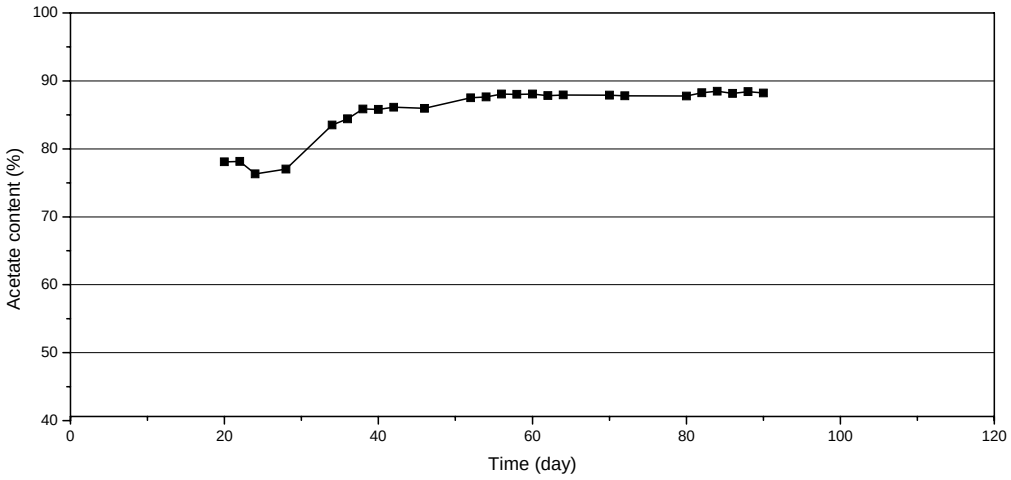


Figure 9-20. Acetate content for air-lime-treated bagasse Fermentation Train TB (ammonium bicarbonate, fresh solid 12 g, fresh liquid 100 mL, and constant cake weight 300 g).

9.5.3 Train TC

Four batch fermentations were initiated by adding 32 g of air-lime-treated bagasse, chicken manure (8 g), ammonium bicarbonate (2 g), nutrient mixture (0.2 g), marine inocula (40 mL), anaerobic water (360 mL), and 120 μL iodoform solution (20 g/L of iodoform dissolved in ethanol). The marine inocula were taken from a previous batch of air-lime-treated bagasse and chicken manure fermentations with ammonium bicarbonate (Chapter V). On each transfer with Train TB, air-lime-treated bagasse (9.6 g), chicken manure (2.4 g), nutrients (0.2 g), and iodoform (120 μL) were added to F1. Nutrients (0.2 g) and iodoform (60 μL) were added to F2, F3, and F4. Ammonium bicarbonate was added to control the pH in the fermentation broth around 7.0 (6.97–7.03). The transfer of solids and liquids was performed as shown in Chapter VII. The transfer of liquids and solids was operated at a two-day interval for Train TB. Fresh anaerobic water (100 mL) was added to F4 on each transfer. The total acid concentration profile and acetate content profile are shown in Figures 9-21 and 9-22.

9.5.4 Summary of air-lime-treated bagasse fermentations

Table 9-3 summarizes the operating conditions for Trains TA, TB, and TC, whereas Table 9-4 shows the results for the countercurrent fermentations. Figure 9-23 lists the mass balance closures for these fermentation trains.

The highest acid productivity of 1.34 g/(L·day) and highest conversion (0.60 g VS digested/g VS fed) occurred at a concentration of 33.71 g/L in Fermentation Train TB (LRT= 25.2 day and VSLR = 4.05 g/(L·day)). The highest selectivity of 0.83 g total acids/g VS digested was in fermentation Train TA (LRT = 31.95 day and VSLR = 4.83 g/(L·day))

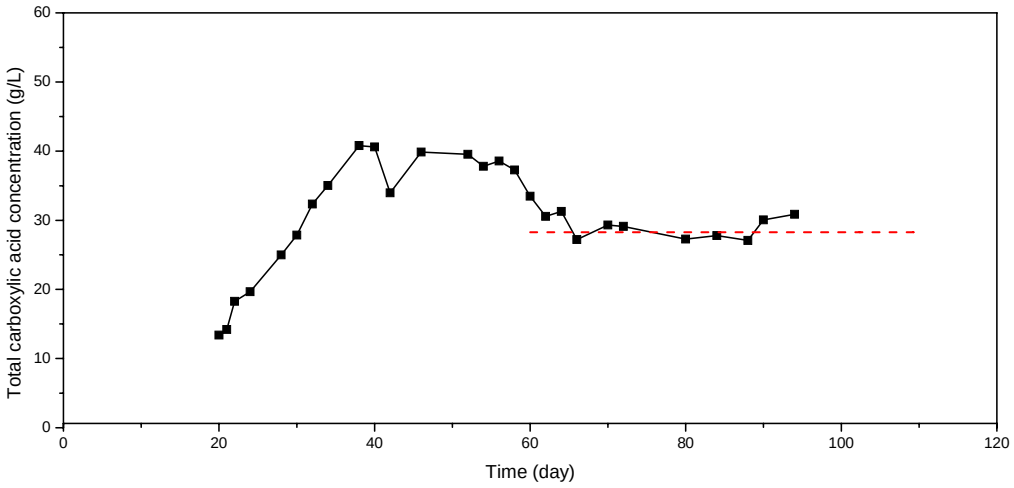


Figure 9-21. Total acid concentration for air-lime-treated bagasse Fermentation Train TC (ammonium bicarbonate, fresh solid 8 g, fresh liquid 100 mL, and constant cake weight 300 g). Dash line indicates steady-state (28.26 g/L).

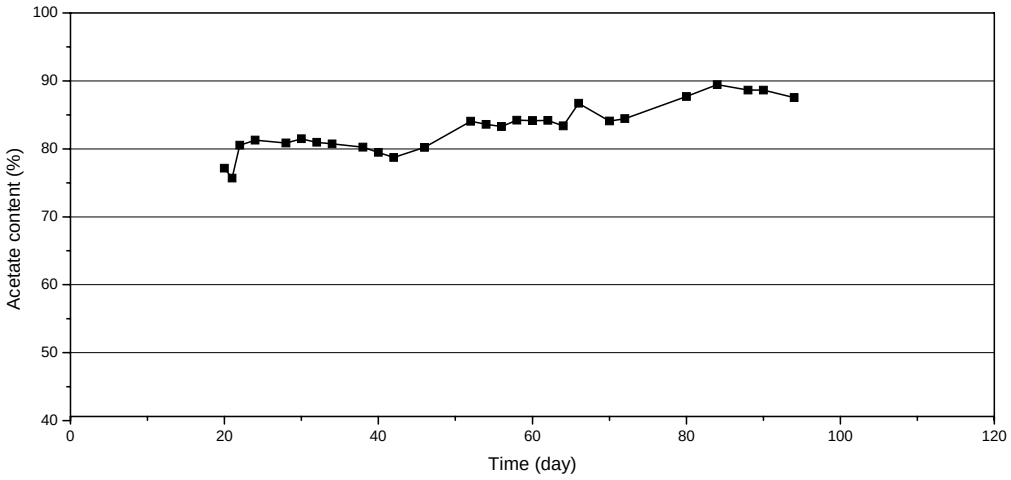


Figure 9-22. Acetate content for air-lime-treated bagasse Fermentation Train TC (ammonium bicarbonate, fresh solid 8 g, fresh liquid 100 mL, and constant cake weight 300 g).

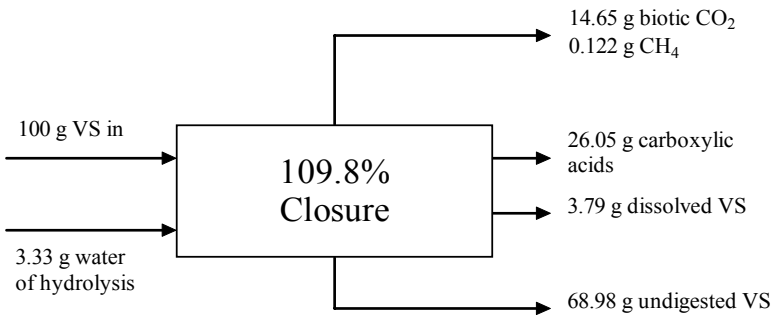
Table 9-3. Operating parameters for air-lime-treated bagasse countercurrent fermentation**.**

Fermentation Trains	TA	TB	TC
LRT (day)	31.95	25.23	23.54
VSLR (g VS/L liquid in all fermentors·day)	4.83	4.05	2.58
VS feed at each transfer (g VS)	11.26	8.45	5.63
Solid feed at each transfer (g)	16.00	12.00	8.00
Treated bagasse (g)	12.80	9.60	6.40
Chicken manure (g)	3.20	2.40	1.60
Liquid fed to F4 at each transfer (L)	0.10	0.10	0.10
VS/liquid feed ratio (g VS/g liquid)	0.11	0.08	0.06
Liquid volume in all four fermentors (L)	1.17	1.04	1.09
Temperature (ºC)	55		
Frequency of transfer	Every two days		
Centrifuge Procedure	Single		
F_1 Retained weight (wet g)	284	288	292
F_2–F_4 Retained weight (wet g)	300	300	300
Iodoform addition rate (mg iodoform added/L liquid fed to F4)	24	24	24
Nutrients addition rate (g dry nutrients added/L liquid fed to F4)	2.0	2.0	2.0
Urea addition rate (g urea added/L liquid feed to F4)	0.0	0.0	0.0

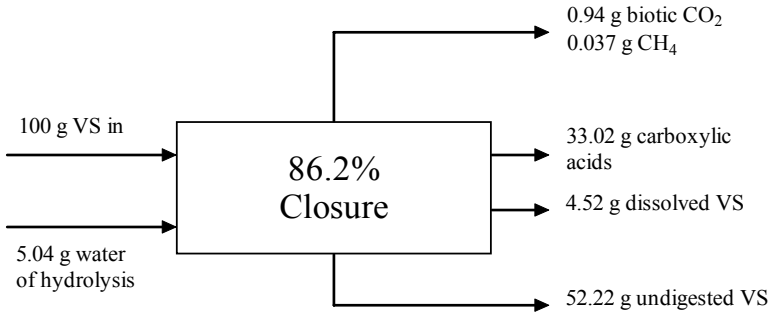
Table 9-4. Fermentation results for air-lime-treated bagasse countercurrent fermentation.

Fermentation Trains	TA	TB	TC
Average pH in all fermentors	6.40±0.37	6.48±0.28	6.56±0.32
Total carboxylic acid concentration (g/L)	40.18±2.16	33.71±0.86	28.26±1.20
Acetic acid (wt%)	87.72±1.06	88.21±0.25	87.09±2.12
Propionic acid (wt%)	2.76±0.11	3.09±0.11	3.02±0.27
Butyric acid (wt%)	9.13±1.00	8.29±0.18	9.45±1.92
valeric acid (wt%)	0.39±0.16	0.40±0.04	0.44±0.21
Caproic acid (wt%)	0.00±0.00	0.00±0.00	0.00±0.00
Heptanoic acid (wt%)	0.00±0.00	0.00±0.00	0.00±0.00
Conversion (g VS digested/g VS fed)	0.31	0.60	0.59
Yield (g total acids/g VS fed)	0.26	0.33	0.47
Selectivity (g total acids/g VS digested)	0.83	0.55	0.79
Total carboxylic acid productivity (g total acids/ (L liquid·day))	1.26	1.34	1.20
Methane productivity (g CH_4/(L liquid·day))	0.0059	0.0015	0.0294
Mass balance closure (g VS out/g VS in)	1.098	0.862	1.147

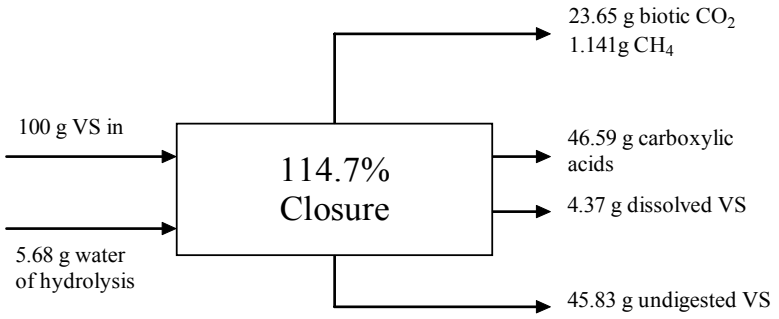
Note: All errors are ± 1 standard deviation.



(a) For Fermentation TA.



(b) For Fermentation TB.



(c) For Fermentation TC.

Figure 9-23. Mass balances for air-lime-treated bagasse Fermentations TA, TB, and TC.

9.6 CPDM prediction

As detailed in Chapter VII, the CPDM method was used to predict the carboxylic acid concentration and conversion for the studied countercurrent fermentation train.

9.6.1 Ammonia-treated bagasse/chicken manure fermentation with ammonium bicarbonate

Batch experiments with ammonia-treated bagasse (80 wt%) and chicken manure (20 wt%) were performed to obtain model parameters for CPDM method, as mentioned in Chapter VII. Sugarcane bagasse was treated with ammonia following the procedure in Appendix B. The marine inoculum for these fermentations was taken from the previous countercurrent Fermentation Train MH, so the microorganisms were already adapted to the substrate. Ammonium bicarbonate was the pH buffer. Liquid samples from the fermentation were analyzed for carboxylic acids. Carboxylic acid concentrations were converted to acetic acid equivalents (Aceq) using Equation 7-11 and Equation 7-12. The Figures 9-24 to 9-28 shows Aceq concentrations for five ammonia-treated bagasse/chicken manure batch experiments. The smooth lines in those figures are the predicted Aceq. Values of the fitted parameters *a*, *b*, and *c* for Equation 7-13 are presented in Table 9-5.

Table 9-5. Values of the parameters *a*, *b*, and *c* fitted by least squares analysis (ammonia-treated bagasse/chicken manure with ammonium bicarbonate).

Initial substrate Concentration (g/L)	*a* (g /L liquid)	*b* (g /(L liquid·d))	*c* (d^{-1})
40	4.39	0.77	0.07
70	4.78	1.33	0.13
100	4.04	3.31	0.11
100+ (a)	23.23	2.43	0.12
100+ (b)	21.48	2.87	0.15

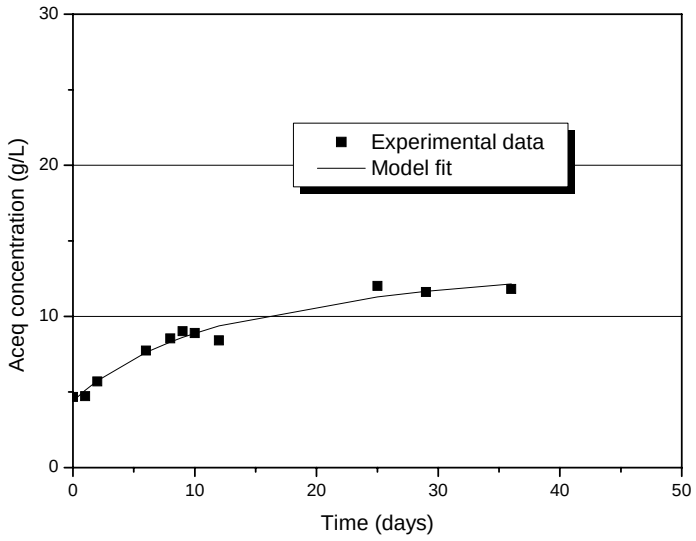


Figure 9-24. Aceq concentration for ammonia-treated bagasse/chicken manure batch fermentor at 40 g substrate /L liquid with ammonium bicarbonate.

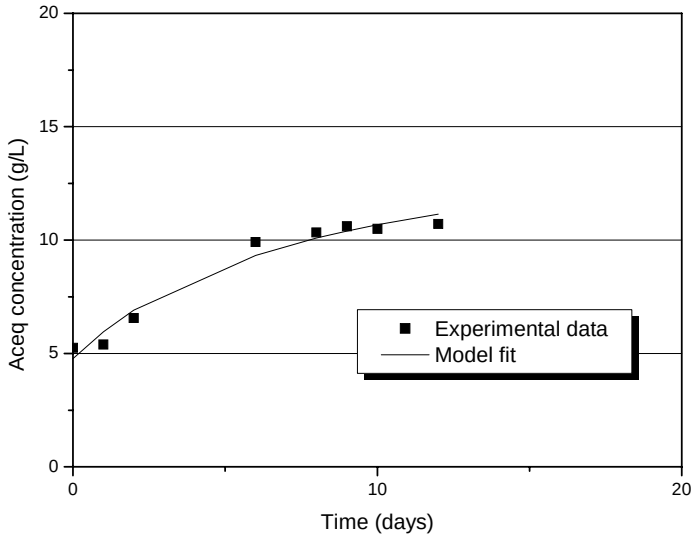


Figure 9-25. Aceq concentration for ammonia-treated bagasse/chicken manure batch fermentor at 70 g substrate /L liquid with ammonium bicarbonate.

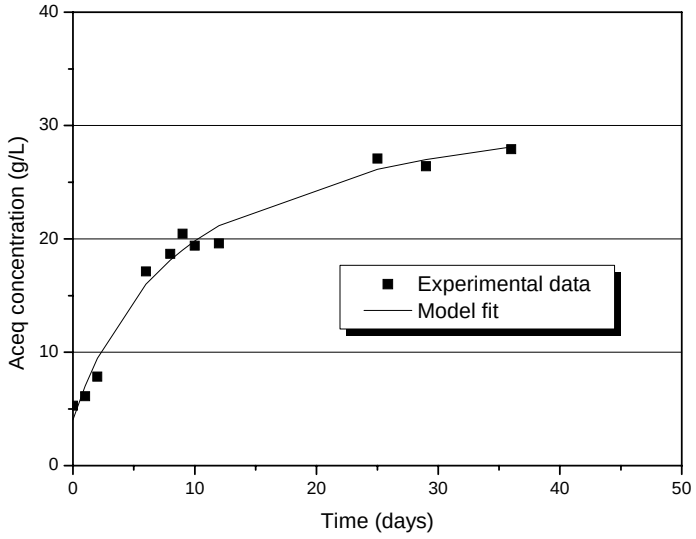


Figure 9-26. Aceq concentration for ammonia-treated bagasse/chicken manure batch fermentor at 100 g substrate /L liquid with ammonium bicarbonate.

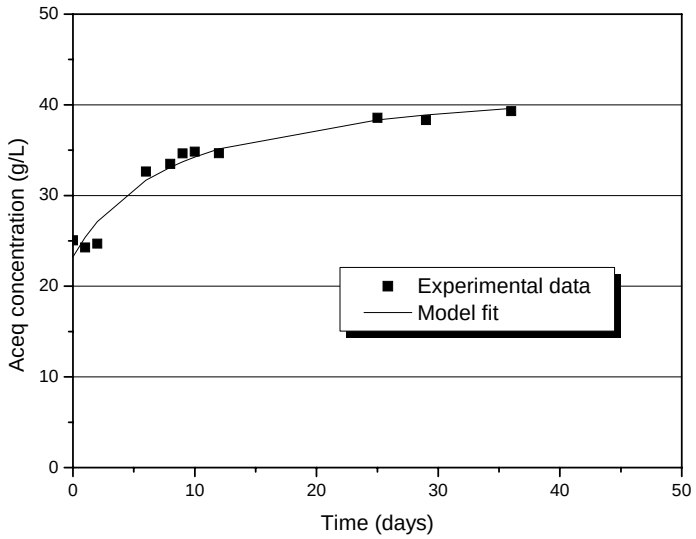


Figure 9-27. Aceq concentration for ammonia-treated bagasse/chicken manure batch fermentor at 100 g substrate + acids (a)/L liquid with ammonium bicarbonate.

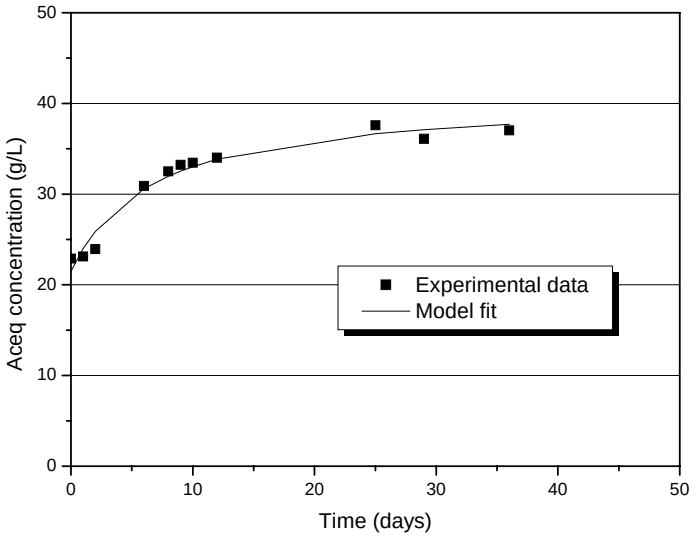


Figure 9-28. Aceq concentration for ammonia-treated bagasse/chicken manure batch fermentor at 100 g substrate + acids (b)/L liquid with ammonium bicarbonate.

The reaction rate and specific reaction rate for batch fermentations were calculated by using Equations 7-15 and 7-16. Conversion was calculated with the experimental acetic acid equivalents using Equation 7-17. Parameters *e*, *f*, *g*, and *h* present in the predicted rate equation (Equation 9-1) were calculated by nonlinear regression (*Systat Sigmaplot 10.0*). Figure 9-29 compares the predicted specific rate with the experimental specific rate. The specific rate equation for the 80 wt% ammonia-treated bagasse/20 wt% chicken manure fermentation with ammonium bicarbonate carbonate follows:

$$\hat{r}_{\text{pred}} = \frac{0.59(1-x)^{3.19}}{1+3.01(\phi \cdot \text{Aceq})^{0.68}} \tag{9-1}$$

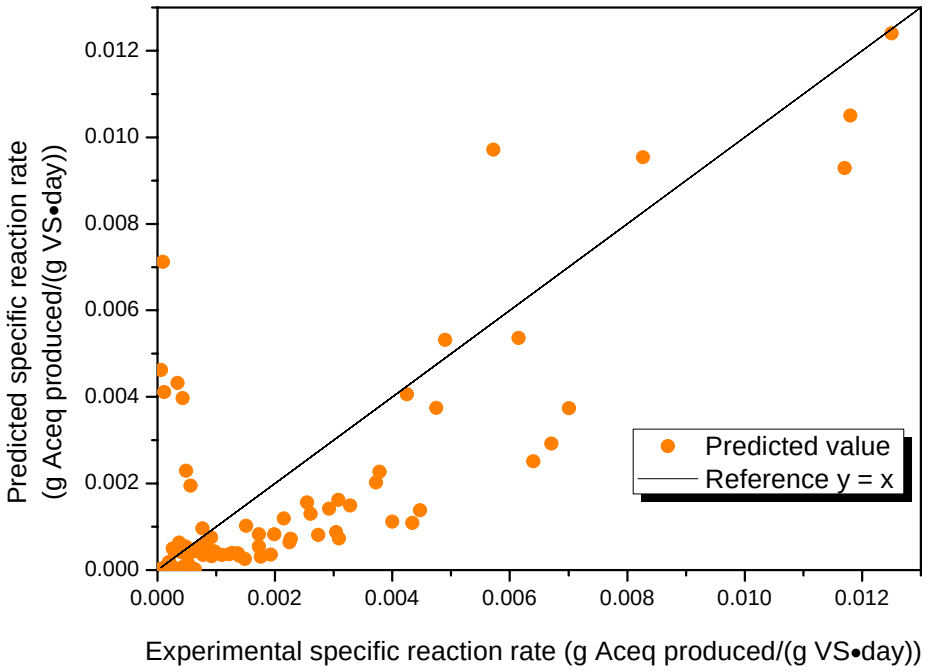


Figure 9-29. The experimental value and the CPDM prediction value for the specific reaction rate in the five batch ammonia-treated bagasse/chicken manure fermentations with ammonium bicarbonate buffer.

Table 9-6. Parameter constant values in CPDM for ammonia-treated bagasse/chicken manure fermentation system with ammonium bicarbonate.

Parameter constant	Value
Holdup (g liquid/g VS cake)	5.64
Moisture (g liquid/g solid feed)	0.03
Selectivity (g Aceq/g VS digested)	0.78
F1–F4 solid concentration (g VS/L)	121
F1–F4 liquid volume (L)	0.293
ϕ (g total acid/g Aceq)	0.89
e (g Aceq/(g VS·d))	1.07
f (dimensionless)	3.88
g (L/g total acid)$^{1/h}$	1.87
h (dimensionless)	0.99

Table 9-6 lists the system-specific variables used for the CPDM prediction, whereas Table 9-7 compares the experimental total carboxylic acid concentration and conversion to the CPDM predictions. As shown in Table 9-7, the total carboxylic acid concentrations from experiments agreed well with the CPDM predicted values, with an average absolute error of 4.44%. Substrate conversions for experimental and predicted conditions were very close, with an average absolute error of 12.49%.

Table 9-7. Comparison of experimental and predicted carboxylic acid concentration for ammonia-treated bagasse/chicken manure fermentations with ammonium bicarbonate.

	Train MH	**Train MK**	**Train ML**	**Train NH**	**Train NL**	**Train NK**	**Average[**] (%)**
Experimental carboxylic acid concentration (g/L)	43.69	35.44	29.79	43.79	27.64	37.03	
Predicted (CPDM) carboxylic acid concentration (g/L)	40.55	35.48	29.78	41.72	31.46	36.74	
Error[*] (%)	-7.18	0.11	-0.05	-4.73	13.81	-0.78	4.44
Experimental conversion	0.41	0.35	0.53	0.40	0.65	0.41	
Predicted (CPDM) conversion	0.34	0.43	0.56	0.35	0.58	0.43	
Error[*] (%)	-18.05	22.00	5.09	-12.50	-11.38	5.93	12.49

* Error (%) = ((Predicted value – Experimental value)/Experimental value) × 100

** Average errors are based on absolute value.

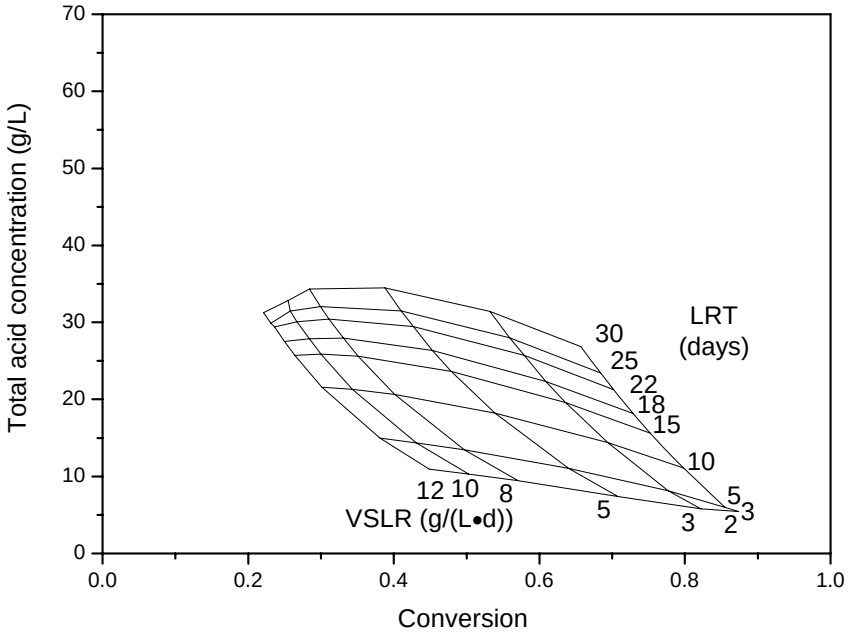


Figure 9-30. The CPDM "map" for 80 wt% ammonia-treated bagasse/20 wt% chicken manure countercurrent fermentation with ammonium bicarbonate (121 g VS/L liquid).

Figure 9-30 shows the CPDM "map" for ammonia-treated bagasse/chicken manure countercurrent fermentation with ammonium bicarbonate at a fermentation solid concentration of 121 g VS/(L of liquid), the average solid concentration in the studied Fermentation Trains MH, MK, ML, NH, NK, and NL. The "map" predicts a total acid concentration of 34.50 g/L at LRT of 30 day, VSLR of 5 g/(L·d) and a conversion of 38.8%. At a VSLR of 2 g/(L·d) and LRT of 3 day, a total acid concentration of 5.43 g/L could be obtained at 86.2% conversion.

9.6.2 Air-lime-treated bagasse/chicken manure with ammonium bicarbonate

Batch experiments with air-lime-treated bagasse (80 wt%) and chicken manure (20 wt%) were performed to obtain model parameters for CPDM method, as mentioned in Chapter VII. Sugarcane bagasse was treated with lime for 2 months following by the procedure in Appendix C. The marine inoculum for these fermentations was taken from countercurrent Fermentation Train TA, so the microorganisms were already adapted to the air-lime-treated bagasse. Ammonium bicarbonate was the pH buffer. Liquid samples from batch fermentations were analyzed for carboxylic acids. Carboxylic acid concentrations were converted to Aceq using Equation 7-11 and Equation 7-12. The Aceq concentrations for the five air-lime-treated bagasse/chicken manure batch experiments are shown in Figures 9-31 to 9-35. The smooth lines in those figures are the predicted Aceq. Values of the fitted parameters *a*, *b*, and *c* for Equation 7-13 are presented in Table 9-8.

$$\text{Aceq} = a + \frac{bt}{1+ct} \tag{7-13}$$

Table 9-8. Values of the parameters *a*, *b*, and *c* fitted by least squares analysis (air-lime-treated bagasse/chicken manure with ammonium bicarbonate).

Initial substrate Concentration (g/L)	*a* (g /L liquid)	*b* (g /(L liquid·d))	*c* (d^{-1})
40	8.73	1.62	0.21
70	9.36	1.83	0.09
100	8.54	3.24	0.09
100+ (a)	25.66	1.70	0.07
100+ (b)	24.49	2.30	0.09

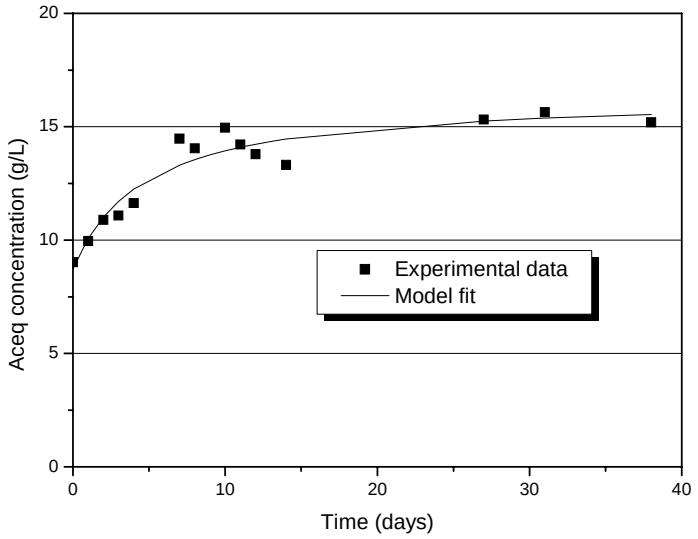


Figure 9-31. Aceq concentration for air-lime-treated bagasse/chicken manure batch fermentor at 40 g substrate /L liquid with ammonium bicarbonate.

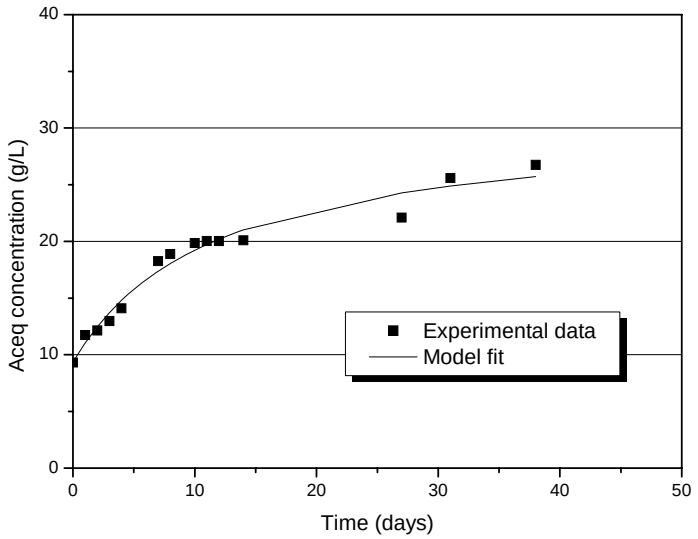


Figure 9-32. Aceq concentration for air-lime-treated bagasse/chicken manure batch fermentor at 70 g substrate /L liquid with ammonium bicarbonate.

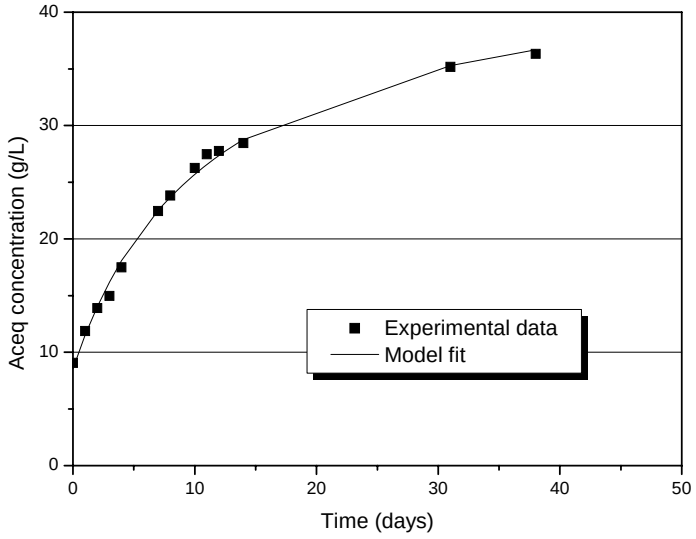


Figure 9-33. Aceq concentration for air-lime-treated bagasse/chicken manure batch fermentor at 100 g substrate /L liquid with ammonium bicarbonate.

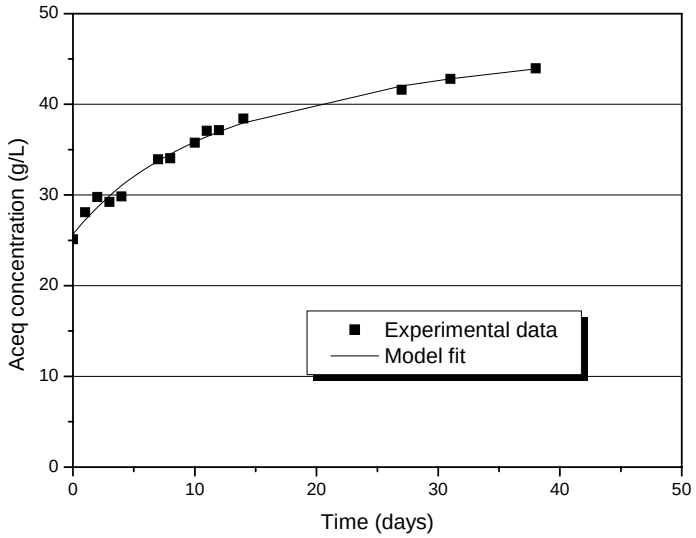


Figure 9-34. Aceq concentration for air-lime-treated bagasse/chicken manure batch fermentor at 100 g substrate + acids (a)/L liquid with ammonium bicarbonate.

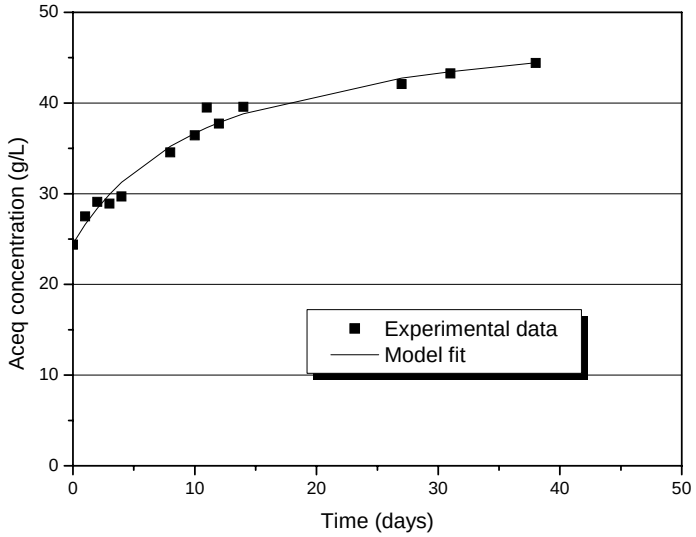


Figure 9-35. Aceq concentration for air-lime-treated bagasse/chicken manure batch fermentor at 100 g substrate + acids (b)/L liquid with ammonium bicarbonate.

The reaction rate and specific reaction rate for batch fermentations were calculated by using Equations 7-15 and 7-16. Conversion was calculated with the experimental acetic acid equivalents using Equation 7-17. Parameters *e*, *f*, *g*, and *h* present in the predicted rate equation (Equation 9-2) were calculated by nonlinear regression (*Systat Sigmaplot 10.0*). Figure 9-36 compares the predicted specific rate with the experimental specific rate. The specific rate equation for the 80 wt% air-lime-treated bagasse/20 wt% chicken manure fermentation with ammonium bicarbonate follows:

$$\hat{r}_{\text{pred}} = \frac{0.71(1-x)^{3.19}}{1+3.09(\phi \cdot \text{Aceq})^{0.68}} \tag{9-2}$$

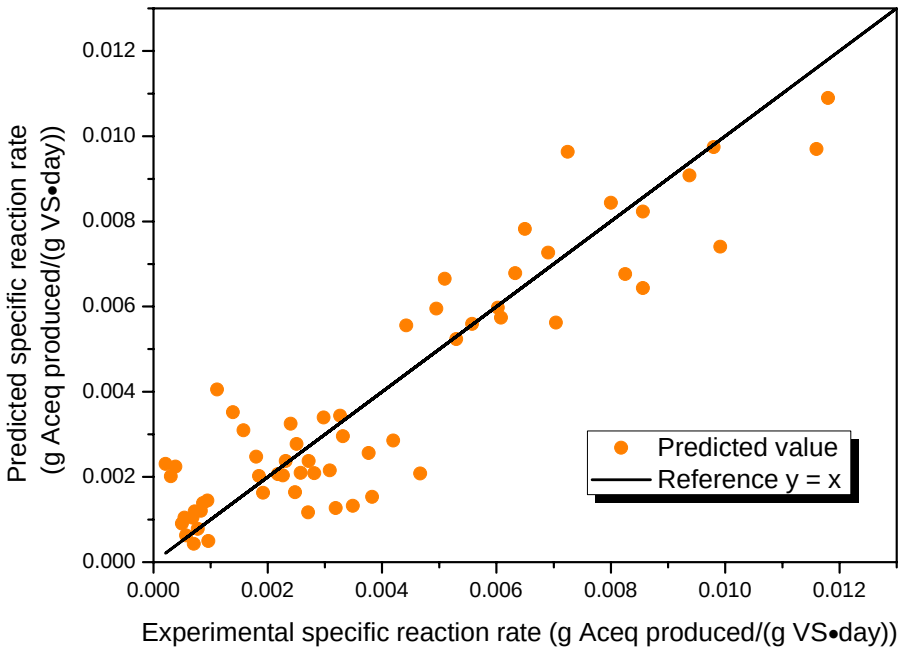


Figure 9-36. The experimental value and the CPDM prediction value for the specific reaction rate in the five batch air-lime-treated bagasse/chicken manure fermentations with ammonium bicarbonate.

Table 9-9. Parameter constant values in CPDM for air-lime-treated bagasse/chicken manure fermentation system with ammonium bicarbonate.

Parameter constant	Value
Holdup (g liquid/g VS cake)	4.02
Moisture (g liquid/g solid feed)	0.03
Selectivity (g Aceq/g VS digested)	0.72
F1–F4 solid concentration (g VS/L)	159
F1–F4 liquid volume (L)	0.275
ϕ (g total acid/g Aceq)	0.90
e (g Aceq/(g VS·d))	0.71
f (dimensionless)	3.19
g (L/g total acid)$^{1/h}$	3.09
h (dimensionless)	0.68

Table 9-9 lists the system-specific variables used for the CPDM prediction, whereas Table 9-10 compares the experimental total carboxylic acid concentration and conversion to the CPDM prediction. As shown in Table 9-10, the total carboxylic acid concentrations from experiments agreed well with the CPDM predicted values, with an average absolute error of 8.53%. Substrate conversion for experimental and predicted value is pretty close, with an average absolute error of 9.77%.

Table 9-10. Comparison of experimental and predicted carboxylic acid concentration for air-lime-treated bagasse/chicken manure fermentations with ammonium bicarbonate.

	Train TA	**Train TB**	**Train TC**	**Average[**] (%)**
Experimental carboxylic acid concentration (g/L)	40.18	33.71	28.26	
Predicted (CPDM) carboxylic acid concentration (g/L)	45.82	37.087	28.69	
Error[*] (%)	14.04	10.02	1.52	8.53
Experimental conversion	0.51	0.60	0.59	
Predicted (CPDM) conversion	0.50	0.58	0.73	
Error[*] (%)	-2.75	-2.83	23.73	9.77

* Error (%) = ((Predicted value – Experimental value)/Experimental value) × 100

** Average errors are based on absolute value.

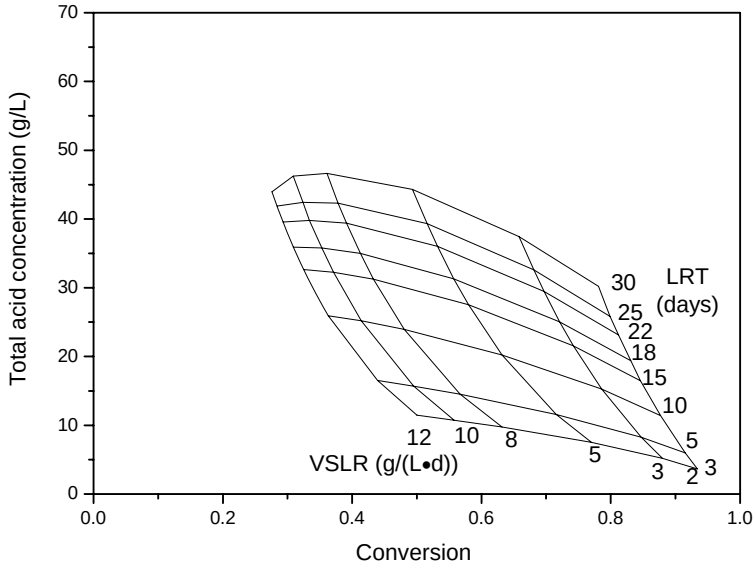


Figure 9-37. The CPDM "map" for 80 wt% air-lime-treated bagasse/20 wt% chicken manure countercurrent fermentation with ammonium bicarbonate (159 g VS/L liquid).

Figure 9-37 shows the CPDM "map" for air-lime-treated bagasse / chicken manure countercurrent fermentation with ammonium bicarbonate at a fermentation solid concentration of 159 g VS/(L of liquid), the average solid concentration in the studied fermentation Train TA, TB, and TC. The "map" predicts a total acid concentration of 46.6 g/L at LRT of 30 day, VSLR of 8 g/(L·d) and a conversion of 36.1%. Relatively high acid concentration (> 30 g/L) and high conversion (>75%) are obtained at a VSLR of 2 g/(L·d) and LRT of 30 day. At a VSLR of 2 g/(L·d) and LRT of 3 day, a total acid concentration of 3.67 g/L could be obtained at 93.4% conversion.

9.7 Summarized comparison of different pretreatment methods

9.7.1 Fermentation performance

Higher substrate concentrations would be allowed if the process was operated on a large scale (Holtzapple et al., 1999). A higher VS concentration should result in higher total carboxylic acid concentrations. CPDM method was used to simulate this industrial fermentor with this high solid concentration of 300 g VS/(L liquid) for both treated bagasse. The acid concentration and conversion of treated bagasse fermentations are illustrated in Figures 9-38 to 9-40.

Figure 9-38 shows fermentation behavior with ammonia-treated bagasse in an industrial scale. As illustrated in the CPDM "map" in Figure 9-38, total acid concentrations as high as 56.46 g/L can be reached at LRT of 30 days, and VSLR of 8 g/(L·d). Also, conversions as high as 96.1% can be achieved at LRT of 2 days and VSLR of 2 g/(L·d). Both high conversions (> 80%) and high product concentrations (> 40 g/L) can be achieved at LRT of 23 days and VSLR 5 g/(L·d).

Figure 9-39 illustrated the air-lime-treated bagasse fermentation. As illustrated in the CPDM "map" of Figure 9-39, total acid concentrations as high as 64.3 g/L can be reached at LRT of 30 days, and VSLR of 10 g/(L·d) for air-lime treated bagasse. Also, conversions as high as 97% can be achieved at LRT of 2 days and VSLR of 2 g/(L·d). Both high conversions (> 75%) and high product concentrations (> 40 g/L) can be achieved at LRT of 30 days and VSLR of 3 g/(L·d).

In conclusion, air-lime-treated bagasse has a better fermentation performance than the ammonia-treated bagasse. Higher conversion and higher acid concentration is achieved in air-lime-treated bagasse fermentation; however, the fermentation difference is not large. This may result from the great performance of ammonium bicarbonate buffer. Ammonium bicarbonate may somehow offset the better performance of air-lime treatment than ammonia treatment.

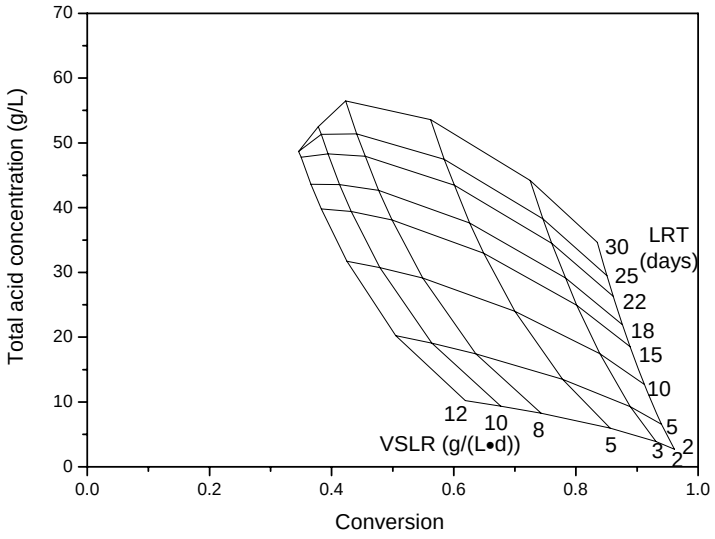


Figure 9-38. The CPDM "map" for 80 wt% ammonia-treated bagasse/20 wt% chicken manure countercurrent fermentation with ammonium bicarbonate buffer (300 g VS/L liquid).

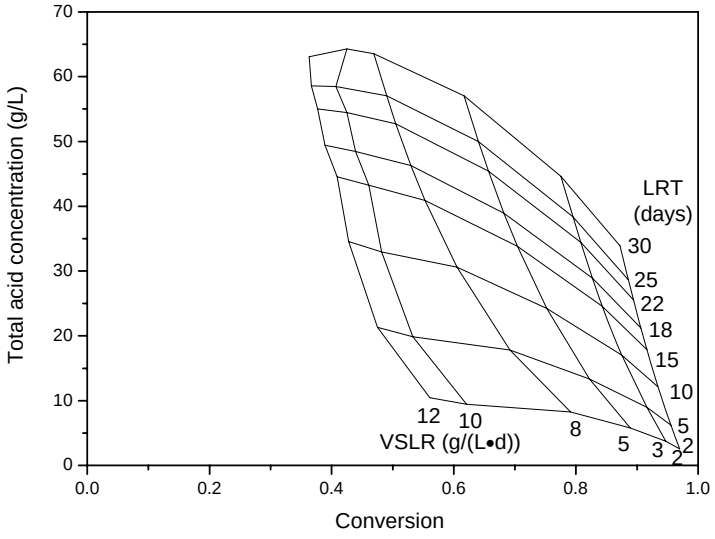


Figure 9-39. The CPDM "map" for 80 wt% air-lime-treated bagasse/20 wt% chicken manure countercurrent fermentation with ammonium bicarbonate buffer (300 g VS/L liquid).

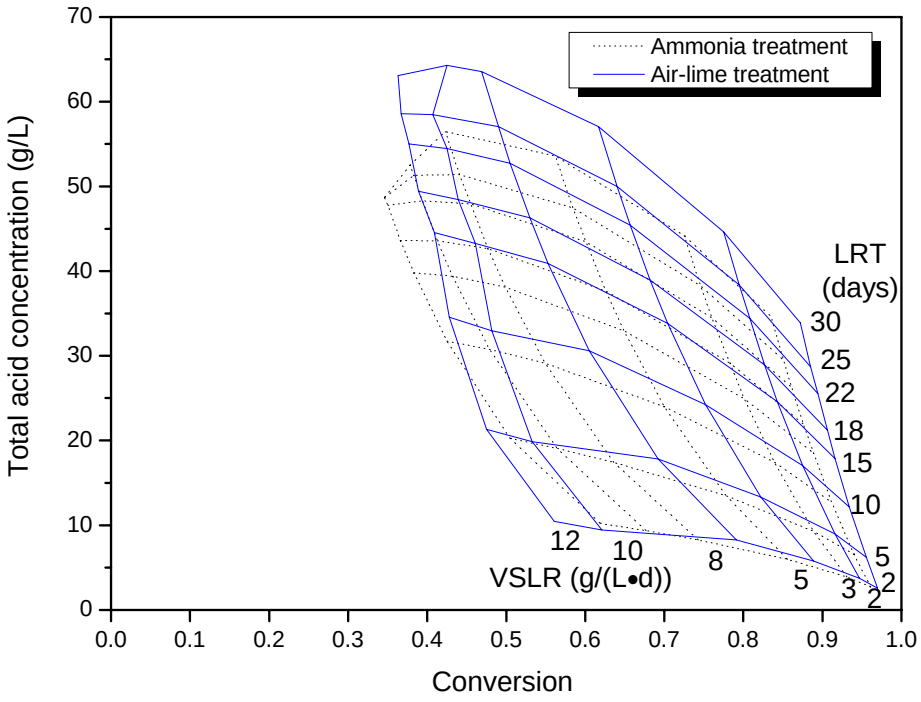


Figure 9-40. The CPDM "map" for 80 wt% treated bagasse/20 wt% chicken manure countercurrent fermentation with ammonium bicarbonate buffer (300 g VS/L liquid). Ammonia treatment and air-lime treatment were used.

9.7.2 Preliminary evaluation of industrial pretreatment methods for ammonium bicarbonate buffered fermentations

As concluded in this dissertation, ammonium bicarbonate is the preferred buffer for anaerobic fermentations in the MixAlco process. An efficient pretreatment method increases the surface area and "accessibility" of the lignocellulosic biomass to anaerobic microorganism. This part attempts to make a preliminary comparison of the three selected biomass treatments (i.e., hot-lime-water treatment, air-lime treatment, and aqueous ammonia treatment).

Table 9-11 compares pretreatment yield for the three studied pretreatment methods. The hot-lime-water treatment (100°C and treatment time of 2 hours) achieved the highest yield of 94.5% in laboratory scale. This results from no washing procedure used in hot-lime-water treatment, causing little biomass lose during pretreatment. Ammonia treatment has lower VS yield (61.96%) than air-lime treatment (74.29%), because ammonia treatment requires several washing.

Lime (144.98 USD/tone) is cheaper than ammonia (224.06 USD/tone) in Table 9-11. Pretreatment chemical cost in ammonia treatment (459.32 USD/tone biomass) is nearly 10 times of that in air-lime treatment (43.49 USD/tone biomass), based on batch pretreatments. However, in industrial application of aqueous ammonia treatment, the cost will be largely decrease due to the possible “ammonia recycle” as mentioned in Section 9.8. Therefore, chemical cost is not a considerable factor in this evaluation.

High temperature (100°C) in hot-lime-water treatment is not preferred in industrial scale, whereas mild temperature (50–55°C) in ammonia treatment and air-lime treatment is desirable. Table 9-11 shows that overall acid yield from air-lime-treated bagasse (0.19 g acid/g dry raw bagasse) is 18.8% higher than ammonia-treated bagasse (0.16 g acid/g dry raw bagasse). Therefore, air-lime treatment is preferred for ammonium bicarbonate buffered fermentation at the industrial scale.

In summary, for the ammonium bicarbonate buffered fermentations, a suitable biomass pretreatment should be evaluated based on pretreatment yield, treatment agent cost, treatment agent recovery, and fermentation yield.

Table 9-11. Effects of different pretreatment methods on ammonium bicarbonate buffered fermentations.

	Chemical usage (g chemical/g dry biomass)	Chemical market price (US$/tonne chemical)[b]	Chemical cost (US$/tonne dry biomass)	Dry weight yield from pretreatment (%)[c]	VS yield from pretreatment (%)[d]	Fermentation yield (g acid/g VS in treated bagasse)[e]	Overall acid yield (g acid/g dry raw bagasse)
Hot-lime-water treatment	0.1	144.98	14.50	94.5	87.79	0.27	0.24
Air-lime treatment	0.3	144.98	43.49	77.5	74.29	0.26	0.19
Ammonia treatment	2.05[a]	224.06	459.32	64.6	61.98	0.26	0.16

[a] 30% ammonia solution with a ratio of 10 mL/g dry raw biomass, where liquid density of ammonia (1.013 bar) is 0.682 g/mL (http://encyclopedia.airliquide.com/encyclopedia.asp?GasID=2).
[b] lime and ammonia market prices refer to http://ed.icheme.org/costchem.html.
[c] Yield = (Dry weight of treated biomass/Dry weight of untreated biomass) × 100
Note: for lime treatment, the dry weight of untreated biomass included dry weight of lime.
[d] VS yield = (Total VS of treated biomass/total VS of untreated biomass) × 100
[e] The fermentation yield was based on Fermentation Trains MD, MK, and TA, respectively.

9.8 Industrial applications

As concluded earlier in this dissertation, ammonium bicarbonate is a better buffer than calcium carbonate. Industrial anaerobic fermentations in the MixAlco process should utilize ammonium bicarbonate as the pH buffer. All biomass pretreatment and fermentation conditions should be optimized to make best use of this newly introduced ammonium bicarbonate buffer. Based on the success of ammonia pretreatment and long-term lime pretreatment, two novel modification of the MixAlco process are therefore proposed as the following based on different biomass feedstock: a) short-time (24 hours) ammonia treatment of biomass, followed by ammonium bicarbonate buffered fermentations; b) for annual harvested biomass feedstock (e.g., crop), long-term lime treatment with air purging is applicable.

9.8.1 The modified MixAlco process combining aqueous ammonia treatment and ammonium bicarbonate buffered fermentations

This process modification integrates ammonia treatment with ammonium bicarbonate buffered fermentations. It aims to recover ammonia and carbon dioxide in "ammonia cycle" and "carbon dioxide cycle."

Process description

Figure 9-41 summarizes the proposed modified MixAlco process combining ammonia pretreatments and ammonium bicarbonate buffered fermentations. Aqueous ammonia solution (NH_3) is used as the pretreatment agents and ammonium bicarbonate (NH_4HCO_3) is the selected buffer agent to adjust the pH in anaerobic fermentations. Raw biomass is pretreated with aqueous ammonia solution to enhance digestibility and fermented anaerobically using the carboxylic acid-forming microorganisms from marine source. The carboxylate salts of ammonium are obtained by adding ammonium bicarbonate buffer. The concentrated salt solution can be processed according to two possible pathways.

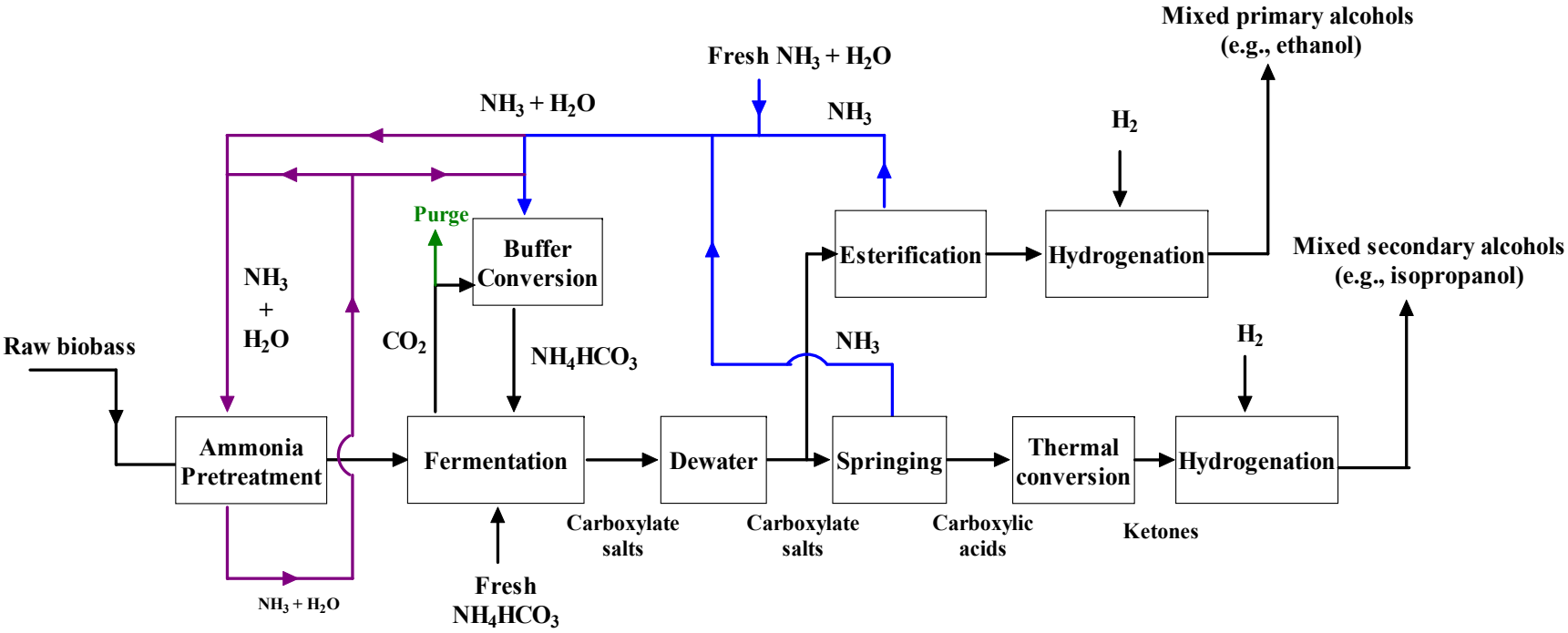


Figure 9-41. Flow diagram of the proposed MixAlco process combining aqueous ammonia pretreatment and ammonium bicarbonate fermentation.

In the first option, the concentrated carboxylate salts can be converted to carboxylic acids by "acid springing"; the acids are further thermally converted to ketones which are further converted to mixed secondary alcohols (e.g., isopropanol) by hydrogenation. In the second option, the concentrated salts can be esterified and then hydrogenated to mixed primary alcohols (e.g., ethanol).

Ammonia cycle and carbon dioxide cycle

The process chemicals are recoverable in this modified process. Ammonia (NH_3) and carbon dioxide (CO_2), intermediate products in the proposed process, are involved in two internal cycles: ammonia cycle and carbon dioxide cycle.

a) Ammonia cycle

Ammonia consumption

- Biomass pretreatment: $NH_3 + H_2O \leftrightarrows NH_3H_2O$
- Buffer conversion: $NH_3 + H_2O + CO_2 \leftrightarrows NH_4HCO_3$

Ammonia feed

- Fresh ammonia solution used for biomass treatment
- Residual aqueous ammonia from biomass treatment process
- Harvested ammonia from acid springing process

$$CH_3(CH_2)_xCOONH_4 \leftrightarrows CH_3(CH_2)_xCOOH + NH_3$$

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

b) Carbon dioxide cycle

Carbon dioxide produced from anaerobic fermentations can be recycled by "buffer conversion process" as shown in Figure 9-41. Carbon dioxide could react with the excess ammonia from the "ammonia input" in ammonia cycle (part a) to produce ammonium bicarbonate. The resulting ammonium bicarbonate is the desired buffer for anaerobic fermentations in the MixAlco process. Alternatively, biotic carbon dioxide,

the metabolic product of microorganisms, could be purged to the air. Because this "biotic portion" of carbon dioxide originates from the adsorbed carbon during photosynthesis, releasing biotic carbon dioxide does not bring new carbon to the atmosphere.

Based on its superior performance, ammonium bicarbonate is chosen as the preferred buffer for fermentations in the MixAlco process. The aqueous ammonia pretreatment in this modified MixAlco process is a good match to ammonium bicarbonate buffer.

One of the benefits could be simplified the downstream product separation. The other highlight of this modified MixAlco process will be the fast and effective ammonia treatment. Experimental results in Chapters IV and V show that 24-hour short-term ammonia treatment at 55°C is sufficient for further fermentation and competitive with the hot-lime-water treatment at 105°C.

The shortcoming of this modified process lies with the higher price of ammonia, compared with lime. However, recovering ammonia in "ammonia cycle" decreases total consumption of ammonia solution. The required sealed treatment reactor in ammonia treatment process is another issue and may also increase capital cost.

In summary, this novel process combined ammonia treatment and ammonium bicarbonate buffered fermentation is feasible.

9.8.2 The modified MixAlco process combining air-lime treatment and ammonium bicarbonate buffered fermentations

In "crop-to-fuel" concept, the ultimate objective is to convert agriculture crops to transportation fuels. Some crops are harvested annually or semi-annually. In this case, the long-term lime treatment will be a promising option. Several months of robust pretreatment will greatly increase crop conversion to carboxylic acids and further fuels.

This modified process is a minor update to the traditional MixAlco process, which combines lime treatment and calcium carbonate buffered fermentations. In this novel modification, no expensive investment in treatment reactors is required; inexpensive and safe lime is deployed; crops are stored in a pretreatment and fermentation pile (Figure 9-42). The stored crops are pretreated with lime (0.3 g $Ca(OH)_2$/g raw biomass) under the optimal conditions (50°C, 8 weeks, and aeration), the fermentation can be performed in the same pile by direct inoculation a mixed culture of marine microorganisms. High product concentration in fermentations is expected to achieve due to the newly introduced ammonia bicarbonate buffer.

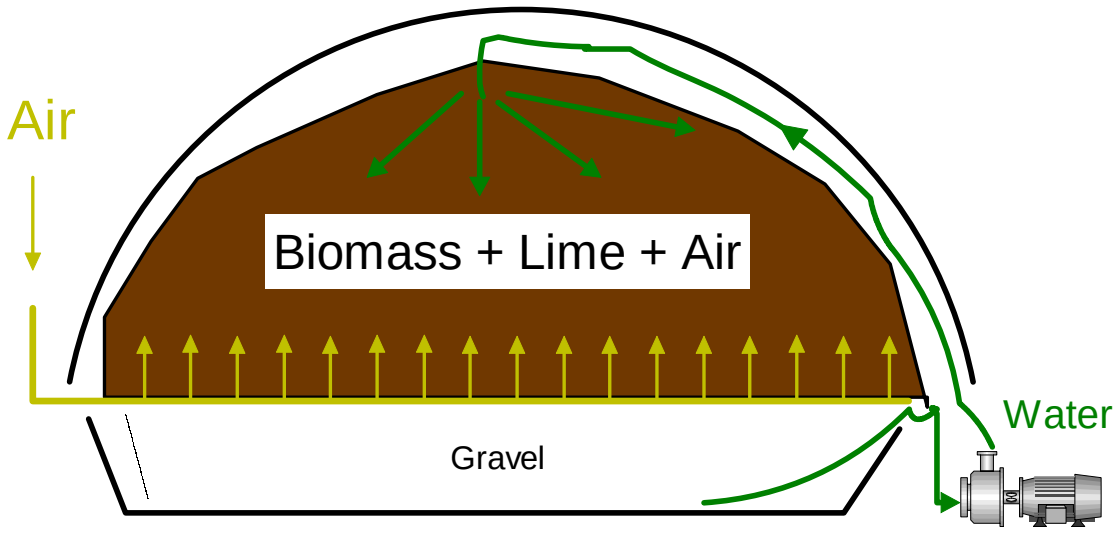


Figure 9-42. Cross-sectional view of treatment and fermentation pile. Air-lime treatment is used. Ammonium bicarbonate is used as buffer in anaerobic fermentations.

9.9 Conclusions

The following conclusions can be made based on the study in this chapter:

1) Air-lime-treated bagasse had a better fermentation performance than ammonia-treated bagasse. There is around 10% higher acid concentration.

2) The modified MixAlco process combined ammonia treatment and ammonium bicarbonate buffered fermentation is recommended if the "ammonia recycle" is deployed in the process.

3) High acid concentration and high conversion is possible in air-lime-treated bagasse fermentations. At a VS concentration of 159 g/L, total carboxylic acid concentrations as high as 46.6 g/L can be reached at LRT of 30 days, and VSLR of 8 g/(L·d) for air-lime treated bagasse. Also, conversions as high as 93.4% can be achieved at LRT of 3 days and VSLR of 2 g/(L·d).

4) For ammonia-treated bagasse, at a VS concentration of 121 g/L, total acid concentrations as high as 34.5 g/L can be reached at LRT of 30 day, and VSLR of 5 g/(L·d). Also, conversions as high as 86.2% can be achieved at LRT of 3 days and VSLR of 2 g/(L·d).

CHAPTER X
CONCLUSIONS AND RECOMMENDATIONS

10.1 Conclusions

Ammonium bicarbonate (NH_4HCO_3) was shown to be a better pH buffer than previously used calcium carbonate ($CaCO_3$) in anaerobic fermentations under thermophilic conditions (55°C). The total product concentrations from paper fermentations using ammonium bicarbonate is almost double that using calcium carbonate, if the pH of ammonium bicarbonate buffered fermentation is maintained around 7.0. There is around 50–60% increase of total carboxylic acid concentration for bagasse fermentations. Acetate content of total carboxylic acids fermented from office paper using ammonium bicarbonate could reach about 92% under thermophilic conditions. This is higher than thermophilic fermentations using calcium carbonate, which were ~70% acetate.

Fermentations buffered by ammonium bicarbonate are pH sensitive. If the pH is 8.0 or above, the product concentration is low. The desired pH range should be controlled within the range of 6.5 to 7.5. Step-wise buffer addition is recommended for ammonium bicarbonate buffer. Further comparison of the ammonium bicarbonate and calcium carbonate under fixed pH conditions show that ammonium bicarbonate is a better buffer. Ammonium bicarbonate is a “weak” methane inhibitor. Around 3% methane was detected in the gas phase of the fermentation system showing that a strong methane inhibitor (e.g., iodoform) is still required in ammonium bicarbonate buffered fermentations.

Aqueous ammonia treatment is a feasible biomass treatment for sugarcane bagasse. Anaerobic fermentations of ammonia-treated bagasse have similar performance as bagasse treated with hot-lime-water treatment, if ammonium bicarbonate is used as the pH buffer. Long-term (12 days) ammonia treatment at room temperature does not exceed the short-term (1 day) treatment in fermentation performance. However, treated bagasse with a higher ammonia concentration (30%) had a better fermentation performance than that with low ammonia concentration (10%).

It has been estimated that around 11.9% weight ratio of residual calcium salts remains in the lime-treated biomass. Residual calcium salts from lime treatment are assumed to have the following potential negative effects: a) mixed buffer effect of calcium carbonate and ammonium bicarbonate, b) biomass blocked by residual calcium salts, and c) toxicity of excess calcium salts residual in fermentation broth. "Simulated lime-treated paper" with additional 11.9% calcium carbonate did not exhibit significant fermentation differences from the original paper substrate. The addition of calcium carbonate did not block the paper micropores and functioned as a pH buffer only. The mixed effect of ammonium bicarbonate and calcium carbonate did not show negative effects on paper fermentations. HCl neutralization and washing could not fully remove the residual calcium salts in the lime-treated biomass. Of the total residual calcium salts (based on metal composition analysis), 13% were difficult to remove by an HCl solution and were assumed to stay in the biomass micropores. Further biomass fermentations showed that the residual calcium salts did not affect ammonium bicarbonate buffered fermentations. Long-term air-lime-treated bagasse achieved best fermentation performance, but it requires a 2-month treatment time.

The lake inocula from the Great Salt Lake, UT worked in the anaerobic fermentation under both thermophilic (55°C) and mesophilic conditions (40°C). Under mesophilic conditions, it had a comparable or better performance than the marine inocula. This confirmed the assumptions that "robust" microorganisms acclimated to the

high salt concentration in the Great Salt Lake may be well suited to the anaerobic fermentations of the MixAlco process. Under mesophilic conditions (40°C), the "brown" inoculum from the Great Salt Lake exceeded the marine inocula, including the original source and an adapted culture. The concentration of total carboxylic acids increased around 30%; however, there was no significant difference between the marine sources and the lake sources under thermophilic conditions (55°C). This is only an explanation if methane was in the lake fermentation, but not the marine fermentation. Thermophilic fermentations (55°C) obtained a higher reaction rate and higher acetic acid percentage compared with mesophilic fermentations (40°C). For the adapted marine inocula, there is no obvious difference in the first 3 weeks of the thermophilic fermentations compared with the mesophilic fermentations. After 3 weeks, some significant differences occurred. On Day 46, the thermophilic fermentation obtained a higher total carboxylic acids concentration of 25.9 g/L compared with 16.4 g/L under mesophilic condition (40°C) for the initial 80 g/L 80% lime-treated bagasse/20% chicken manure. A higher acetic acid percentage 85% was achieved at 55°C, compared with 75% at 40°C.

Fermentation results based on long-term countercurrent fermentations showed that anaerobic microorganisms from the marine source (sediments from different locations in Galveston Island, TX) could adapt to ammonium bicarbonate buffer. Stable acid concentrations were achieved during 330 days of fermentation. The CPDM method is a powerful tool to predict product concentration and conversion based on batch fermentation data. The experimental acid concentration and conversion agree well with the CPDM prediction (average absolute error < 15%) in the countercurrent fermentations.

Ammonium bicarbonate proved to be a better buffer than calcium carbonate in long-term hot-lime-water-treated bagasse countercurrent fermentations. For ammonium bicarbonate buffered fermentation at a VS concentration of 130 g/L, a total acid concentration of 43.42 g/L was achieved at LRT of 30 day, VSLR of 10 g/(L·d), and a conversion of 41.1%. At a VSLR of 3 g/(L·d) and LRT of 3 day, a total acid

concentration of 3.72 g/L could be obtained at 90.2% conversion. For calcium carbonate at a VS concentration of 124 g/L, a total acid concentration of 20.53 g/L was achieved at LRT of 30 day, VSLR of 8 g/(L·d), and a conversion of 34.0%. At a VSLR of 2.5 g/(L·d) and LRT of 3 day, a total acid concentration of 2.47 g/L could be obtained at 92.9% conversion.

High acid concentration and high conversion is possible in air-lime-treated bagasse fermentations. At a VS concentration of 159 g/L, total carboxylic acid concentrations as high as 46.6 g/L can be reached at LRT of 30 days, and VSLR of 8 g/(L·d) for air-lime treated bagasse. Also, conversions as high as 93.4% can be achieved at LRT of 3 days and VSLR of 2 g/(L·d). For ammonia-treated bagasse, at a VS concentration of 121 g/L, total acid concentrations as high as 34.5 g/L can be reached at LRT of 30 day, and VSLR of 5 g/(L·d). Also, conversions as high as 86.2% can be achieved at LRT of 3 days and VSLR of 2 g/(L·d).

Air-lime treatment coupled with ammonium bicarbonate is recommended, but it requires long-term treatment (~2 months). The modified MixAlco process combined ammonia treatment and ammonium bicarbonate buffered fermentation is also feasible if "ammonia recycle" is deployed.

10.2 Future work

Future research should focus on better understanding in better pH control, mesophilic fermentations, microbiologic features, and hydrogen production from fermentations. The objective is to improve pretreatment and fermentation conditions so that the MixAlco process could be cost competitive with traditional fossil fuels.

10.2.1 Automatic ammonium bicarbonate addition to control pH

pH is critical condition for stability and performance of anaerobic fermentations. Most of anaerobic fermentations in this dissertation utilized batch addition of ammonium bicarbonate buffer. Batch addition of buffer is necessary for laboratory countercurrent fermentation, because of the limit in fermentors and incubator. At the pilot scale, automatic pH control is needed for real-time feeding of ammonium bicarbonate. More investigations of pH control in the laboratory can provide support for pilot performance and help the application of ammonium bicarbonate into the MixAlco process.

10.2.2 Mesophilic fermentations using ammonium bicarbonate buffer

One of major differences between thermophilic fermentations and mesophilic fermentations is the product distribution (e.g., acetate content). Thermophilic fermentations yield higher percentages of acetic acids, which benefits ethanol production. In another case, higher molecular weight (HMW) carboxylic acids may be desired. Long-term countercurrent fermentations under mesophilic conditions are expected to verify the assumption of high C4–C6 percentages.

Compared to terrestrial microorganisms, the use of marine inoculum was a breakthrough for the MixAlco process (Aiello Mazzarri 2002). Microorganisms from marine sources work in ammonium bicarbonate buffered fermentations. Even better, lake inoculum from the Great Salt Lake is better than marine inoculum under mesophilic conditions (Chapter VI). Further investigation on lake inoculum under mesophilic conditions is expected to have better fermentation performance than marine inoculum.

10.2.3 Microbiologic feature of anaerobic microorganisms

Better performance in microorganisms (from marine inocula to lake inocula) and buffer (from calcium carbonate to ammonium bicarbonate) indicate that fundamental research on biological features of the mixed culture of microorganism could be fruitful. The objectives follow: a) to identify specific organisms that are robust and grow best in ammonium bicarbonate buffered fermentations; b) to recycle microorganisms from the fermented biomass and mix them with fresh biomass; therefore, nutrient requirements may be reduced.

10.2.4 Hydrogen production from fermentations

As described in Chapter I, hydrogenation is required to convert intermediate products to final mixed alcohols in the MixAlco process. An inexpensive source for hydrogen is one of our interests. Purchasing hydrogen will increase the final product cost. Preliminary paper fermentations showed approximately 10–20% hydrogen in the fermentation effluent gas.

A crucial question surrounds the best balance for producing both carboxylic acids and hydrogen. Are there better fermentation conditions for hydrogen, if carboxylic acids are still expected high production in fermentation? What is the role of ammonium bicarbonate in hydrogen production? In conclusion, hydrogen production from anaerobic fermentation could be a good hydrogen source for the MixAlco process.

REFERENCES

Adjaye JD, Sharma RK, Bakhshi NN. 1992. Characterization and stability analysis of wood-derived bio-oil. Fuel Processing Technology 31(3):241-256.

Agbogbo F. 2005. Anaerobic Fermentation of Rice Straw and Chicken Manure to Carboxylic Acids [Dissertation]. College station, TX: Texas A&M University.

Aiello Mazzarri C. 2002. Conversion of Municipal Solid Waste to Carboxylic Acids by Anaerobic Countercurrent Fermentation [Dissertation]. College station, TX: Texas A&M University.

Backreedy RI, Fletcher LM, Jones JM, Ma L, Pourkashanian M, Williams A. 2005. Co-firing pulverised coal and biomass: A modeling approach. Proceedings of the Combustion Institute 30:2955-2964.

Bernardo A, Howard-Hildige R, O'Connell A, Nichol R, Ryan J, Rice B, Roche E, Leahy JJ. 2003. Camelina oil as a fuel for diesel transport engines. Industrial Crops and Products 17(3):191-197.

Castro MBG, Remmerswaal JAM, Reuter MA. 2003. Life cycle impact assessment of the average passenger vehicle in the Netherlands. International Journal of Life Cycle Assessment 8(5):297-304.

Chan WN, Holtzapple MT. 2003. Conversion of municipal solid wastes to carboxylic acids by thermophilic fermentation. Applied Biochemistry and Biotechnology 111(2):93-112.

Chang VS, Burr B, Holtzapple MT. 1997. Lime pretreatment of switchgrass. Applied Biochemistry and Biotechnology 63-5:3-19.

Chang VS, Nagwani M, Holtzapple MT. 1998. Lime pretreatment of crop residues bagasse and wheat straw. Applied Biochemistry and Biotechnology 74(3):135-159.

Chang VS, Nagwani M, Kim CH, Holtzapple MT. 2001. Oxidative lime pretreatment of high-lignin biomass - Poplar wood and newspaper. Applied Biochemistry and Biotechnology 94(1):1-28.

Claassen PAM, van Lier JB, Contreras AML, van Niel EWJ, Sijtsma L, Stams AJM, de Vries SS, Weusthuis RA. 1999. Utilisation of biomass for the supply of energy carriers. Applied Microbiology and Biotechnology 52(6):741-755.

Culcuoglu E, Unay E, Karaosmanoglu F. 2002. Rapeseed cake as a biomass source. Energy Sources 24(4):329-336.

David P. Chynoweth RI. 1987. Anaerobic Digestion of Biomass. New York: Technology & Industrial Arts. 296 p.

DeJager D, Blok K. 1996. Cost-effectiveness of emission-reducing measures for methane in the Netherlands. Energy Conversion and Management 37(6-8):1181-1186.

Demirbas A. 2003. Biomass co-firing for coal-fired boilers. Energy Exploration & Exploitation 21(3):269-278.

Demirbas A. 2005. Biomass co-firing for boilers associated with environmental impacts. Energy Sources 27(14):1385-1396.

Demirbas MF, Balat M. 2006. Recent advances on the production and utilization trends of bio-fuels: A global perspective. Energy Conversion and Management 47(15-16):2371-2381.

Dien BS, Cotta MA, Jeffries TW. 2003. Bacteria engineered for fuel ethanol production: Current status. Applied Microbiology and Biotechnology 63(3):258-266.

Domke SB, Aiello-Mazzarri C, Holtzapple MT. 2004. Mixed acid fermentation of paper fines and industrial biosludge. Bioresource Technology 91(1):41-51.

Dowaki K, Mori S, Fukushima C, Asai N. 2005. A comprehensive economic analysis of biomass gasification systems. Electrical Engineering in Japan 153(3):52-63.

Faaij A. 1999. Bioenergy and sustainable development. Biofutur 1999(195):16-19.

Gandi J, Holtzapple MT, Ferrer A, Byers FM, Turner ND, Nagwani M, Chang SS. 1997. Lime treatment of agricultural residues to improve rumen digestibility. Animal Feed Science and Technology 68(3-4):195-211.

Gardner N, Manley BJW, Pearson JM. 1993. Gas emissions from landfills and their contributions to global warming. Applied Energy 44(2):165-174.

Gnansounou E, Dauriat A. 2005. Ethanol fuel from biomass: A review. Journal of Scientific & Industrial Research 64(11):809-821.

Goldemberg J. 2000. World energy assessment: Energy and the challenge of sustainability. New York: United Nations Development Programme.

Gordon AS, Austin TC. 1992. Alternative fuels for mobile transport. Progress in Energy and Combustion Science 18(6):493-512.

Granda CB. 2004. Sugarcane Juice Extraction and Preservation, and Long-term Lime Pretreatment of Bagasse [Dissertation]. College station, TX: Texas A&M University.

Granda CB, Holtzapple MT. 2006. Experiences with raw thin sugarcane juice preservation. International Sugar Journal 108(1288):209.

Hansen TL, Sommer SG, Gabriel S, Christensen TH. 2006. Methane production during storage of anaerobically digested municipal organic waste. Journal of Environmental Quality 35(3):830-836.

Hawkins S, Samaj J, Lauvergeat V, Boudet A, GrimaPettenati J. 1997. Cinnamyl alcohol dehydrogenase: Identification of new sites of promoter activity in transgenic poplar. Plant Physiology 113(2):321-325.

Himmel ME, Adney WS, Baker JO, Elander R, McMillan JD, Nieves RA, Sheehan JJ, Thomas SR, Vinzant TB, Zhang M. 1997. Advanced bioethanol production technologies: A perspective. Fuels and Chemicals from Biomass 666:2-45.

Holtzapple MT, Davison RR, Ross MK, Aldrett-Lee S, Nagwani M, Lee CM, Lee C, Adelson S, Kaar W, Gaskin D and others. 1999. Biomass conversion to mixed alcohol fuels using the MixAlco process. Applied Biochemistry and Biotechnology 77-9:609-631.

Holtzapple MT, Loescher M, Ross M, Rapier R, Ghandi J, Burdick S. 1996. Biomass conversion to mixed alcohols. Abstracts of Papers of the American Chemical Society 211:29-Btec.

Holtzapple MT, Ross MK, Chang NS, Chang VS, Adelson SK, Brazel C. 1997. Biomass conversion to mixed alcohol fuels using the MixAlco Process. Fuels and Chemicals from Biomass 666:130-142.

Hsu TA, Ladisch MR, Tsao GT. 1980. Alcohol from cellulose. Chemtech 10(5):315-319.

Iyer PV, Wu ZW, Kim SB, Lee YY. 1996. Ammonia recycled percolation process for pretreatment of herbaceous biomass. Applied Biochemistry and Biotechnology 57-8:121-132.

Jones M. 2007. Effects of Physical and Chemical Pretreatments on the Crystallinity of Bagasse [Dissertation]. College station, TX: Texas A&M University.

Joseph F. Malina, George F, Pohland PB. 1992. Design of anaerobic processes for treatment of industrial and muncipal waste. Boca Raton, FL: CRC Press.

Kamm B, Kamm M. 2004. Principles of biorefineries. Applied Microbiology and Biotechnology 64(2):137-145.

Karaosmanoglu F. 2000. Biobriquetting of rapeseed cake. Energy Sources 22(3):257-267.

Katagiri M, Nakamura M. 2002. Animals are dependent on preformed alpha-amino nitrogen as an essential nutrient. Iubmb Life 53(2):125-129.

Kim S, Holtzapple MT. 2005. Lime pretreatment and enzymatic hydrolysis of corn stover. Bioresource Technology 96(18):1994-2006.

Kim S, Holtzapple MT. 2006a. Delignification kinetics of corn stover in lime pretreatment. Bioresource Technology 97(5):778-785.

Kim S, Holtzapple MT. 2006b. Effect of structural features on enzyme digestibility of corn stover. Bioresource Technology 97(4):583-591.

Kim SH. 2004. Lime Pretreatment and Enzymatic Hydrolysis of Corn Stover [Dissertation]. College station, TX: Texas A&M University.

Kim TH, Kim JS, Sunwoo C, Lee YY. 2003. Pretreatment of corn stover by aqueous ammonia. Bioresource Technology 90(1):39-47.

Kim TH, Lee YY. 2005a. Pretreatment and fractionation of corn stover by ammonia recycle percolation process. Bioresource Technology 96(18):2007-2013.

Kim TH, Lee YY. 2005b. Pretreatment of corn stover by soaking in aqueous ammonia. Applied Biochemistry and Biotechnology 121:1119-1131.

Kim TH, Lee YY, Sunwoo C, Kim JS. 2006. Pretreatment of corn stover by low-liquid ammonia recycle percolation process. Applied Biochemistry and Biotechnology 133(1):41-57.

Kirschenbaum L, J. Kirschenbaum, Grunwald E. 1972. Introduction to Quantitative Chemical Analysis. Englewood Cliffs, NJ: Prentice-Hall. 450 p.

Knauf M, Moniruzzaman M. 2004. Lignocellulosic biomass processing: A perspective. International Sugar Journal 106(1263):147-150.

Kumar A, Bhattacharya SC, Pham HL. 2003. Greenhouse gas mitigation potential of biomass energy technologies in Vietnam using the long range energy alternative planning system model. Energy 28(7):627-654.

Lagerkvist A. 1995. The landfill gas activity of the IEA bioenergy agreement. Biomass & Bioenergy 9(1-5):399-413.

Lee GW, Lee SJ, Jurng J, Hwang J. 2003. Co-firing of paper sludge with high-calorific industrial wastes in a pilot-scale nozzle-grate incinerator. Journal of Hazardous Materials 101(3):273-283.

Lin Y, Tanaka S. 2006. Ethanol fermentation from biomass resources: Current state and prospects. Applied Microbiology and Biotechnology 69(6):627-642.

Lopez R, Poblano VM, Licea-Claverie A, Avalos M, Alvarez-Castillo A, Castano VM. 2000. Alkaline surface modification of sugar cane bagasse. Advanced Composite Materials 9(2):99-108.

Maclean HL. 2004. Alternative transport fuels for the future. International Journal of Vehicle Design 35(1-2):27-49.

Mao T, Show KY. 2006. Performance of high-rate sludge digesters fed with sonicated sludge. Water Science and Technology 54(9):27-33.

Miao XL, Wu QY. 2004. High yield bio-oil production from fast pyrolysis by metabolic controlling of Chlorella protothecoides. Journal of Biotechnology 110(1):85-93.

Moletta R. 2005. Winery and distillery wastewater treatment by anaerobic digestion. Water Science and Technology 51(1):137-144.

Morgan DL. 1947. The Great Salt Lake. New York: The Bobbs-Merrill company. 432 p.

Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology 96(6):673-686.

Mufson S. 2007. Ethanol Production Booming on Demand. http://www.washingtonpost.com/wp-dyn/content/article/2007/01/22/AR2007012201306.html

Murphy JD, McKeogh E, Kiely G. 2004. Technical/economic/environmental analysis of blogas utilisation. Applied Energy 77(4):407-427.

Murphy JD, Power NM. 2006. A technical, economic and environmental comparison of composting and anaerobic digestion of biodegradable municipal waste. Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering 41(5):865-879.

Naber JE, F. Goudriaan, A.S. Louter. 1997. Further development and commercialisation of the small scale hydro-thermal upgrading process for biomass liquefaction. Proceedings of the Third Biomass Conference of the America's. Montreal.

Nguyen PHL, Kuruparan P, Visvanathan C. 2007. Anaerobic digestion of municipal solid waste as a treatment prior to landfill. Bioresource Technology 98(2):380-387.

Ozcimen D, Karaosmanoglu F. 2004. Production and characterization of bio-oil and biochar from rapeseed cake. Renewable Energy 29(5):779-787.

Patnaik P. 2002. Handbook of Inorganic Chemicals. New York: McGraw-Hill Professional. 1086 p.

Peterson JBD. 2006. Ethanol production from agricultural residues. International Sugar Journal 108(1287):177-180.

Ross MK, Holtzapple MT. 2001. Laboratory method for high-solids countercurrent fermentations. Applied Biochemistry and Biotechnology 94(2):111-126.

Stabnikova E, Ang SS, Liu XY, Ivanov V, Tay JH, Wang JY. 2005. The use of hybrid anaerobic solid-liquid (HASL) system for the treatment of lipid-containing food waste. Journal of Chemical Technology and Biotechnology 80(4):455-461.

Steinberg M. 1999. Fossil fuel decarbonization technology for mitigating global warming. International Journal of Hydrogen Energy 24(8):771-777.

Tengerdy RP, Szakacs G. 2003. Bioconversion of lignocellulose in solid substrate fermentation. Biochemical Engineering Journal 13(2-3):169-179.

Thanakoses P. 2002. Conversion of Bagasse and Corn Stover to Mixed Carboxylic Acids Using a Mixed Culture of Mesophilic Microorganisms [Dissertation]. College station, TX: Texas A&M University.

Thanakoses P, Mostafa NAA, Holtzapple MT. 2003. Conversion of sugarcane bagasse to carboxylic acids using a mixed culture of mesophilic microorganisms. Applied Biochemistry and Biotechnology 105:523-546.

Turkenburg W. 2002. Renewable energy technologies. In: J. Goldemberg, Editor, World Energy Assessment: Energy and the Challenge of Sustainability—An Overview. New York: United Nations Development Programme.

Turn SQ. 1999. Biomass integrated gasifier combined cycle technology: Application in the cane sugar industry. International Sugar Journal 101(1205):267.

Vaitilingom G. 2006. Cottonseed oil as biofuel. Cahiers Agricultures 15(1):144-149.

Van Groenestijn J, Hazewinkel O, Bakker R. 2006. Pretreatment of lignocellulose with biological acid recycling (Biosulfurol process). Zuckerindustrie 131(9):639-641.

Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee YY. 2005. Coordinated development of leading biomass pretreatment technologies. Bioresource Technology 96(18):1959-1966.

Yoon HH, Wu ZW, Lee YY. 1995. Ammonia-recycled percolation process for pretreatment of biomass feedstock. Applied Biochemistry and Biotechnology 51-2:5-19.

Zaldivar J, Roca C, Le Foll C, Hahn-Hagerdal B, Olsson L. 2005. Ethanolic fermentation of acid pre-treated starch industry effluents by recombinant Saccharomyces cerevisiae strains. Bioresource Technology 96(15):1670-1676.

Zhang Q, Chang J, Wang TJ, Xu Y. 2007. Review of biomass pyrolysis oil properties and upgrading research. Energy Conversion and Management 48(1):87-92.

APPENDIX A
HOT-LIME-WATER PRETREATMENT PROCEDURE

Lignocellulosic biomass (e.g., sugarcane bagasse) was treated with calcium hydroxide (i.e., lime) in the presence of water in a metal tray. The ground biomass and calcium hydroxide (0.1 g/g dry biomass) were placed in the metal tray and thoroughly mixed. Enough distilled water was added to the dry mixture to cover the material. The tray was then covered with aluminum foil and boiled with Bunsen burners for 2 h. Once the mixture had boiled, it was allowed to cool to room temperature overnight.

1. In a stainless steel pan, place the preweighed biomass, lime, and distilled water. The loadings are 0.1 g of Ca $(OH)_2$/g dry biomass and 10 mL of distilled water/g dry biomass. It is helpful to add the distilled water in two or three batches and to knead the liquid into the biomass after each addition.
2. Mix the three components very thoroughly to ensure even distribution of the lime and water through the biomass. It is helpful to mix the lime in one of the water batches.
3. Place the pan over two Bunsen burners and heat to boiling. Boil the mixed slurry for 2 h and stir occasionally. Add more distilled water if it evaporates.
4. Allow the mix to cool down to room temperature (this takes more than 5 h, usually overnight).
5. Add more distilled water to the mixture to cover the biomass once the mixture is cooled. Add 10 drops of Dow Corning silicone antifoam solution to prevent foaming. Bubble CO_2 through the mixture using diffusing stones to neutralize the lime.
6. Continue to bubble CO_2 until the pH falls below 7.0 throughout the biomass. Mix occassionally. This step may take several hours.
7. Place the pan in the drying oven at 105°C, and allow the mixture to dry. It may takes 2 days. The dried biomass is usually a solid cake. Crumble the solid cake into pieces by hand and store it in a labeled container.

APPENDIX B
AIR-LIME PRETREATMENT PROCEDURE

A pile of biomass (e.g., sugarcane bagasse) was lime pretreated for a maximum of 8 weeks according to the desired conditions (Holtzapple et al. 1999). Approximately 5 kg dry weight of bagasse was mixed with the preweighted 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 a constant temperature of 50°C. Air was scrubbed through lime slurry container and then bubbled through the pile via air diffusers beneath the pile.

Procedure

1. Mix a large amount of raw bagasse (e.g., 5 kg) with excess lime (0.3 g $Ca(OH)_2$/g dry biomass). Mix well to ensure a complete contact between lime and bagasse.
2. Form a pile on top of the rock bed with the bagasse and lime mixture in the storage bin. Pay attention to the amount of the bagasse. The dome covering will not seal properly, if the bin is overloaded.
3. Place the dome covering on top of the 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 tank with water.
7. Control the air valve connected to diffusers located beneath the pile and to maintain air flowing speed around 20 standard cubic feet per hour.
8. Make sure the return line valve to the sump is open, and the valve to the water sprayer is initially closed.
9. Prime both centrifugal pumps.
10. Turn on pumps. 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 set to a temperature of 50°C.

13. Open and adjust the sprayer valve to the appropriate position to be sure water is discharging from each sprinkler onto the pile.
14. Add more water to the sump every other day to maintain a constant water level.
15. Monitor the pH of the lime slurry to ensure basic conditions are maintained.
16. Monitor the pH of the sump weekly to determine when to end the pretreatment (e.g., desired pH of 9).

Check the system daily for leaks, and monitor the strainer in the sump pump discharge line weekly to be sure it is not clogged. The pretreatment is finished when the lignin content is reduced by 50% or when the pH drops below 9, whichever comes first. Shut down the pretreatment after 8 weeks if neither of these conditions occurs before then. Flush the system thoroughly with fresh water before using it again. This may need 6–7 complete flush procedures.

APPENDIX C
AMMONIA PRETREATMENT PROCEDURE

Lignocellulosic biomass (e.g., sugarcane bagasse) was treated with ammonia solution to enhance digestibility. "Long-term" and "short-term" ammonia treatments were used. A self-constructed high-pressure reactor (Figure 4-8) is the desired reactor for short-term treatment. Mild treatment temperature (55^{o}C) was maintained within a modified temperature-adjustable oven (Figure 4-7) or a 1-L centrifuge bottle (Figure 4-10) in short-term ammonia treatment. Long-term treatment only used 1-L centrifuge bottle (Figure 4-10). A roller system (Figure 4-9) created mixing for the long-term treatment. No temperature control was required in the long-term ammonia treatment.

"Short-term" ammonia treatments for batch fermentations

1. Measure desired raw bagasse and the desired volume of ammonia solution, fill them to a homemade high-pressure reactor (Figure 4-8) inside the hood. Make sure to handle ammonia solution inside hood.
2. Close and tight each reactor using PTFE thread seal tape.
3. Load all of the six reactors to the iron supporter and affix it to the self-constructed temperature-controlled oven (Figure 4-7).
4. Control the oven to desired temperature; allow 10 minutes for the oven to reach the desired temperature.
5. Use the variable autotransformer to control the motor rotating speed. Set to 22 volts to maintain the six reactors rotating at a smooth and slow speed.
6. "Cook" or heat the biomass slurry for 1 day.
7. Remove the reactor supporter from the oven; cool the reactors to room temperature to ensure decreasing gas phase pressure in the reactors and avoid possible explosion.
8. Unload the six reactors from the iron supporter in the hood.
9. Collect the biomass to the alumni foil, which was placed on top of a metal tray. Place the metal dry in the hood to air-dry the biomass mixture then followed by a vacuum dry. This is used to remove the ammonia mixed in the biomass.

10. Harvest the air-dried bagasse from the metal tray. The dried biomass is ready for fermentation now.

"Short-term" ammonia treatments for countercurrent fermentations

1. Measure desired raw bagasse and the desired volume of ammonia solution, fill them to a 1-L centrifuge bottle (Figure 4-10) inside the hood.
2. Close and tight each centrifuge bottle.
3. Load the centrifuge bottles in the fermentation incubator (Figure 2-3).
4. "Cook" the biomass mixture at 55^{o}C for 1 day. Frequently check the ammonia pretreatment reactors. Tight the centrifuge bottle, if the top cover of centrifuge bottles becomes loosed.
5. Move the centrifuge bottles to the hood.
6. Cool the centrifuge bottles to room temperature to ensure decreasing gas phase pressure in the reactors and avoid possible explosion.
7. Start the "mix-stir-centrifuge-mix cycle" (Chapter V) until the pH or color of the liquid in the centrifuge bottle remained unchanged (six washes, on average).
8. Centrifuge the treated biomass at 4,000 rpm for 25 minutes. The residual wet solid cake was removed from the centrifuge bottle and dried in the oven at 105^{o}C for at least 2 days.

"Long-term" ammonia treatments for batch fermentations

1. Measure desired raw bagasse and the desired volume of ammonia solution, fill them to a 1-L centrifuge bottle (Figure 4-10) inside the hood.
2. Close and tight each centrifuge bottle.
3. Load the centrifuge bottles in the roller system (Figure 4-9).
4. Treat the biomass mixture for 12 days.
5. Move the centrifuge bottles to the hood.
6. Cool the centrifuge bottles to room temperature to ensure decreasing gas phase pressure in the reactors and avoid possible explosion.
7. Start the "mix-stir-centrifuge-mix cycle" (Chapter V), until the pH or color of the liquid in the centrifuge bottle remained unchanged (e.g., six cycles).
8. Centrifuge the treated biomass at 4,000 rpm for 25 minutes. The residual wet solid cake was removed from the centrifuge bottle and dried in the oven at 105^{o}C for at least 2 days.

APPENDIX D
LIQUID MEDIA PREPARATION

The liquid media used in all fermentation experiments was deoxygenated water with cysteine hydrochloride and sodium sulfide.

1. Fill distilled water into a large glass container (6 L). Place the container over a Bunsen burner to boil. To save time, it is helpful to cover the top with an inverted beaker.
2. Boil distilled water under a nitrogen purge for 5 min.
3. Cool the boiled water to room temperature under nitrogen purge.
4. Add 0.275 g cysteine hydrochloride and 0.275 g sodium sulfide per liter of 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
COUNTERCURRENT TRANSFER PROCEDURES

Liquid and solid flowed in the opposite directions in the countercurrent fermentations. A typical countercurrent train is made up of four fermentors. For a laboratory-scale countercurrent transfer, the transfer of liquid and solids is made every 1, 2, or 3 days, operating in a semi-continuous manner. Countercurrent fermentations were initiated as batch fermentations. The experiments were performed in a batch mode until the culture established in the fermentor (7–10 days). After the culture developed, the countercurrent operation was started, and the liquid and solids were transfer using the single-centrifuge procedure (Figure E-1). To maintain anaerobic conditions in the fermentors, a nitrogen purge should be utilized every time the fermentors are open to the atmosphere.

The single-centrifuge procedure is detailed below and illustrated in Figures E-2 and E-3.

1. Remove the fermentors from the incubator and allow cooling for 10 minutes at room temperature.
2. Release and record the gas production using the device illustrated in Figure 2-7.
3. Remove the fermentor caps and place a nitrogen purge line in the fermentor. Using another nitrogen line, remove the residual solids adhered to the stopper and metals bar and returned to the fermentor.
4. Measure and record pH for each fermentor.
5. Cap the fermentor with a regular centrifuge cap.
6. Balance each pair of the fermentors using some additional weight supplements (e.g., preweighed paper or metal piece). Pay attention to balance the centrifuge bottles before placing it into the centrifuge.
7. Centrifuge the fermentors to separate the solid and the liquid. Centrifuge time varies with the substrate systems. A time of 25 min was preferred for the bagasse/chicken manure system. Centrifuge rotating speed was selected as 4000 rmp and centrifuge brake level was set as 5.

8. After centrifuging, carefully move the bottles to ensure that the solids and liquid do not remix. For the calcium carbonate buffered fermentation, the fermentors can be inverted to keep the liquid in the bottom. For ammonium bicarbonate buffered fermentation, the bottles cannot be inverted because in general the wet cake will loosen and fall.
9. Place the liquid from Fermentor 1 (F1 in Figure E-1) into a previously weighed plastic graduate cylinder. Record the weight and volume of liquid.
10. Take a 4-mL liquid sample for carboxylic acids analysis. Decant the remaining liquid from F1 into a liquid collection bottle for further VS analysis. Store the sample and collection bottle in a freezer for future analysis.
11. Weigh the fermentor with the remaining solids and compare against the goal weight. Remember that the regular centrifuge cap is not included in this weight. To achieve a steady state, a constant wet cake weight must be maintained in each fermentor, and then each fermentor is maintained at a specific weight. If the fermentor weight (wet solids + centrifuge bottle without cap) weighs more than the goal weight, remove the difference aside and the solids will be added to the next fermentor (F2 in Figure E-1). To simplify the transfer calculations, the goal weight includes the desired wet cake weight plus the weight of fresh biomass to be added to F1.

    Example:

    Weight of F1 + wet solids cake = 355 g

    Predetermined wet cake weight = 300 g

    Solids removed from F1= 55 g

12. Pour the liquid from F2 into F1.
13. Add fresh biomass to F1 according to the determined loading rate. Add calcium carbonate, urea, dry nutrients and methane inhibitor. Mix well, replace the stopper and cap the fermentor.

14. Weigh the wet solids from F2. Remove the solids resulting of:

    Solid removed = (F2 wet solids + solids from F1) − the goal weight.

    Example:

    Solids from F1: 55 g

    Weight of F2 + wet solids cake = 265 g

    Predetermined wet cake weight = 275 g

    Solids removed from F2 = 45 g

15. Pour the liquid from Fermentor 3 (F3 in Figure E-1) into F2, and repeat Step 9.
16. Repeat Steps 10 and 11 for F3 and Fermentor 4 (F4 in Figure E-1).
17. Add fresh liquid medium (Appendix D) to F4 according to predetermined volume.
18. Place the solids removed from F4 in a solid collection bottle and store it in the freezer until the VS analysis is performed.
19. Return all fermentors back to the incubator.

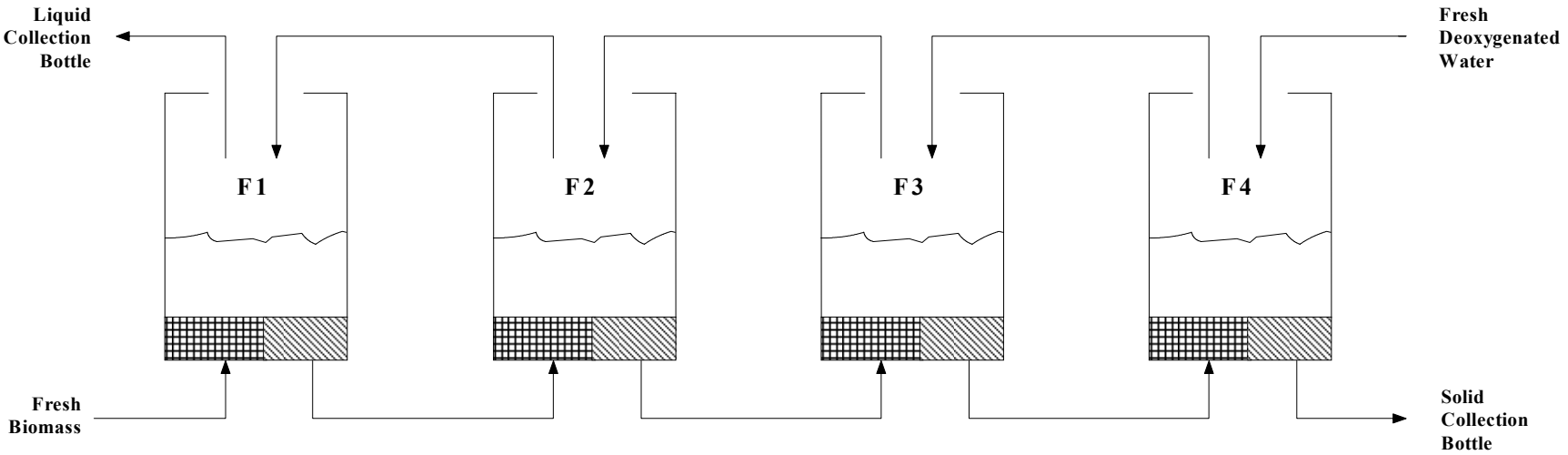


Figure E-1. Single-centrifuge countercurrent procedure.

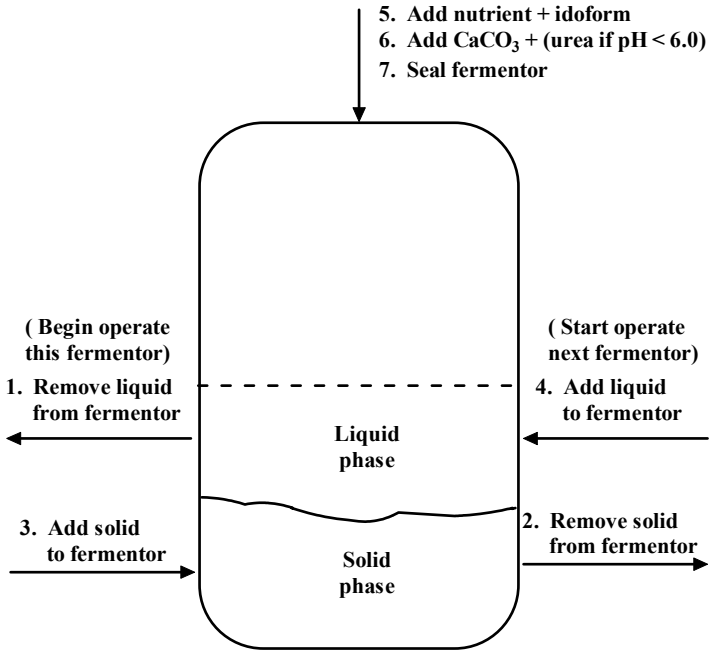


Figure E-2. Countercurrent procedure for calcium carbonate fermentation.

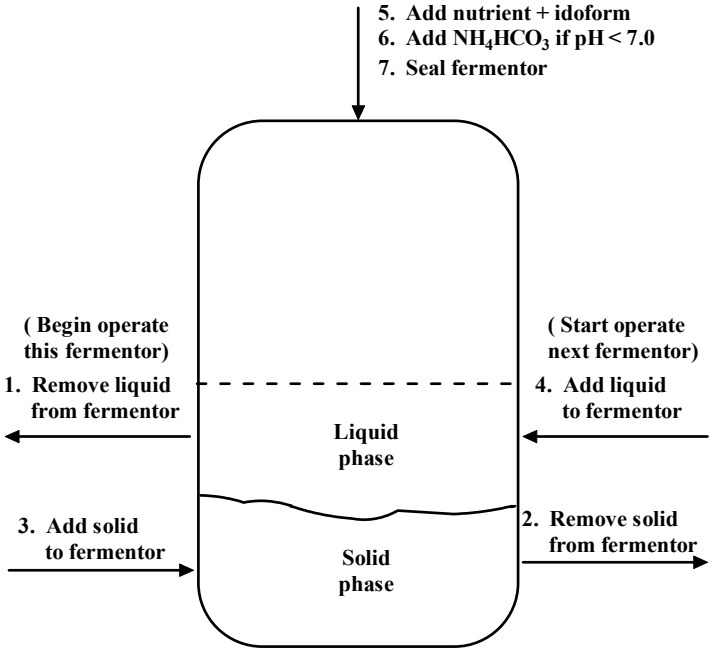


Figure E-3. Countercurrent procedure for ammonium bicarbonate fermentation.

APPENDIX F
CARBOXYLIC ACIDS ANALYSIS

For carboxylic acids analysis, at least 3 mL of liquid should be withdrawn from the fermentor, and placed in a 15-mL conical bottom centrifuge tube. If the samples were not analyzed inmediately, they were stored in the freezer at −15°C. At the moment of the analysis, if the sample was stored in the freezer, defrost and vortex the sample before beginning the procedure. If the acid concentration of the samples is high, they may require further dilution (e.g., 50 vol% sample/50 vol% water) before the standard "GC liquid sample preparation" method mentioned as the following.

GC LIQUID SAMPLE PREPARATION

1. Centrifuge the liquid sample for 5 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-valeric acid (1.162 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 25ºC. Due to the poor refrigeration system in this centrifuge machine, simply accelerate the centrifuge rotating speed to 15,000 rpm and inmediately turn to zero rpm.
7. Remove the round-bottom ultracentrifuge tube and pipette 1 mL of the centrifugated mixture into a glass GC vial and cap the 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 run 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. When finish running the sequence, turn the GC on standby status 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 bottle (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 bottle.
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/liquid product mix into Crucible A. Record the weight of the Crucible A + liquid mix.
8. Dry the crucible at 105°C for two 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 the weight.

13. Mix the remaining content in 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

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

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

$$\mathrm{VS}_{\text{solid reidue}}\,(\mathrm{g\ VS}) = \frac{(\mathrm{W14}-\mathrm{W15})}{\left(\frac{\mathrm{W13}-\mathrm{W15}}{\mathrm{W10}-\mathrm{W16}}\right)}$$

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

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

PROCEDURE FOR SOLID RESIDUE

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 100 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 to cool 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

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

The amount of VS in one gram of collected solid is calculated as

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

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. The constant values for the system-specific parameters are denoted with "**". VSLR and LRT are the independent variables for constructing the CPDM "map."

```
holdup = 1.4;                    **weight ratio of liquid in wet cake (g liquid/g VS in wet cake)**
moist =0.08;                     **weight ratio of liquid in biomass feed  (g liquid/g VS in feed)**
so = 0.6;                        **selectivity, σ (g Aceq/g VS digested)**
ratio = 0.84;                    **ratio of g total acid to g Aceq**
stages = 4;
loading =6;                      **VSLR**
tauloverall = 15;                **LRT**
vol = {  .17,  .17,  .17,   .17};  **individual liquid volume in fermentors (L)**
totvol = Sum[vol[[i]],{i,1,stages}];
liquidfeed = totvol/tauloverall;
nnotreal = {150,150,150,150};;   **VS concentration in fermentors (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->1.66,f->1.28, g->3.22, h->0.396};                    **CPDM parameters**

**The following codes do not require modification, if you are not sure**

   rmodel[x_,acd_]:=e (1-x)^f/(1+g (acd*ratio)^h)/.fit;
   rmodel[x,acd];
   slp=D[rmodel[x,ac],x];
   drmodel[xx_,aac_]:=slp/.{x    xx,ac    aac};
   drmodel[x,ac];


   acid={30,20,15,5};
   ans=Table[1,{i,1,stages}];
   tauloverallnew=20;
   taulnew=Table[1000,{i,1,stages}];
   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,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}];
    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["------------------------------------------------------------------------"];
    Print["Total Aceq concentration in each stage  ",acid ];
    Print["Total carboxylic acid concentration in each stage  ",acid ratio];
    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",Convrsn];
    Print["------------------------------------------------------------------------"];
    Print["VSLR =     ",loading    , " g VS/L.day"];
   Print["LRT =   ",tauloverall , " day"];



    Print["**** CPDM prediction is: ****"];
    Print["Total carboxylic acid concentration in 1st fermentor (F1):   ",acid[[1]] ratio   , " g/L"];
    Print["Conversion in last Fermentor (F4):  ",Convrsn[[4]]];
    Print["------------------------------------------------------------------------"];
    Print["VSLR =     ",loading    , " g VS/L.day", "    LRT =   ",tauloverall , " day;  ",  "Total carboxylic
acid concentration in F1:   ",acid[[1]] ratio   , " g/L   and conversion in F4:", Convrsn[[4]] ];
```

APPENDIX I

CPDM MATLAB PROGRAM

```
% ========================================================================
%   Improved MATLAB Code for CPDM prediction
% - This source code is for a standard four-stage countercurrent fermentation
% - Program is used to predict acid concentration and conversion at varying VSLR and LRT.
% - This code was modified and tested by Zhihong Fu on 10/05/2004
%  Department of Chemical Engineering, Texas A&M University, College Station, TX
% ========================================================================

clear all
close all
global so taus a1 b1 c1 d1 e1 f1
global holdup moist ratio stages loading tauloverall
global ratio acid nnot factr1
global x_1 nhat_1 x_2 nhat_2 x_3 nhat_3 x_4 nhat_4


%% Record result to Local file
diary off;
YESNO='';
while isempty(YESNO)
    YESNO = input('Do you want to diary the result? Y/N [Y]: ','s');
end
if strcmpi(YESNO , 'Y') == 1
   M5 = clock;
   disp(['For example, you can put: ', num2str(M5(2:4), '%2i-'),num2str(M5(5), '%2i'),'.txt']);
   resultfile='';
   while isempty(resultfile)
       resultfile = input('Input the file name, default path is MATLAB path: ','s');
   end
   diary( num2str(resultfile) );
end


%% Start Simpulation
disp(['Program starts at:  ',  datestr(now)]) ;
tic;


VSLR_data=[3, 4 ,6 ,8 ,12]';
LRT_data=[5, 10, 15 ,25, 30, 35]';
VSLR_loop=3.5;   % k loop is for varing VSLR, (Volatile solids loading rates )
while VSLR_loop<3.51

LRT_loop = 1; % L1 loop is for varing LRT (Liquid residence time)
while LRT_loop < 1.01
```

```
%% Basic parameter for Fermentation.
stages = 4;  % Fermentor stages

so = 0.45;    % total acid selectivity (g aceq produced/g VS digested)
% - Based on Dr. Chan, P120
% - selectivity can be obtained from the keyboard input also.
%so = input('Input total acid selectivity , (default is 0.8):   ');

holdup = 2.0;     % ratio of liquid to solid in wet cake  (g liquid/g VS cake)
moist =0.06;    % ratio of liquid to solid in feed ((g liquid/g VS cake))
SQ = 1.0;
ratio = 0.9;  % ϕ ratio of g total acid to g ACEQ
loading =6;   % VSLR   (g VS/L Liquid/day)
tauloverall =15*LRT_loop; %LRT
vol = [.48,.24,.24,.24]';  %Liquid volume in fermentors
totvol = sum(vol);
liquidfeed = totvol/tauloverall;
nnotreal = [169,214,214,214]';   %VS concentration  g VS/L)
solidfeed = loading * totvol ;    % Solid Feed (g dry weight)
Convrsn = [.1,.2,.3,.4]';  % Initial value for conversion
nnot = nnotreal./(1-Convrsn);
taus = nnot.*vol/solidfeed;
L=0.1*ones(stages+1,1);  % L initial value for liquid flow rate in every reactor.
taul = tauloverall/stages*ones(stages,1); %taul = Table[tauloverall/stages, {i, 1, stages}];


% **********************************
%  Regression of the Equations
%  Disabled in this source code.
% **********************************
mgm1 = 3;
if mgm1 == 100
disp('Regression reaction equations.....');
fid = fopen('exp.txt','w');
fprintf(fid,'%6.2f  %12.8f\n',y);
fclose(fid);
load count.dat
% create the  matrix count in the workspace. For this example, extract the first column of traffic counts
and assign it to the vector x.
x111 = count(:,1);
end


a1=0.07;b1=6.42;c1=0.0;d1=0.0;e1=6.42;f1=1.33;   % CPDM model Parameters
%acd=22.3;  % acd need to transfe into the Function M file
rmodel = @(x1,acd) a1.*(1-x1).^b1./(1+c1.*(10.*x1).^d1+e1.*(acd.*ratio).^f1);
syms x1 acd
drmodel_1 = diff(a1.*(1-x1).^b1./(1+c1.*(10.*x1).^d1+e1.*(acd.*ratio).^f1),x1);
drmodel = @(x2,acd2) subs(drmodel_1,{x1,acd},{x2,acd2});

done = 0;    % The index used to trace whether the condition is satisfied

liqtoler = 0.005;   % tolerance for Liquid Flowrate
```

```
acidtoler = 0.02;  % tolerance for acid concentration
nnottoler = 1;    % tolerance for nnot


% Initial values for acid, acidold
ans=ones(stages,1);
acid =[30,20,15,5]';
acidold = ones(stages,1);
taulnew = 1000*ones(stages,1);   %Column Vector
nhatzero =100*ones(stages,1) ;   % Continuum particle concentration
creation = ones(stages,1);
destruction = ones(stages,1);
tauloverallnew=20;

disp('Calculation is in progress.....');

while done < 0.50
    taulnew = 1000*ones(stages,1);
    % Obtain Flowrate for each fermentor
    taulover_error = 0.001 ;
    while abs(tauloverall-tauloverallnew) > taulover_error
      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);
    end

   taul = vol./L(1:stages) ;   % vol 4*1 , L 5*1
   nnot = nnotreal./(1-Convrsn);
   taus = nnot.*vol/solidfeed;
   scale = ones(stages,1);

   disp(['    nnot=     ', num2str(nnot', '%15.5f')]);

   % parameter for ODE45
   options = odeset('RelTol',1e-4,'AbsTol',1e-4);
   x_low=0; x_high=0.99;
   %==================================
   %      %%  Reactor 1      %%%
   %==================================
   i=1;
   while  abs(taulnew(i) - taul(i)) > liqtoler     %liqtoler=0.05
      nhat0=nhatzero(i);
      [x,nhat] = ode15s(@chan1,[x_low,x_high],nhat0,options);
      x_1=x;nhat_1=nhat;
      F_1 = @(x_1)interp1(x,nhat,x_1) ;
      factr1 = nnot(i)/quad(F_1,x_low,x_high) ;  % claculate factor
      F_11 = @(x_1) factr1*interp1(x,nhat,x_1).*rmodel(x_1,acid(i)) ;
      robs = quad(F_11,x_low,x_high) ;   %
      F_12 = @(x_1) interp1(x,nhat,x_1).*x_1 ;
```

```
    Convrsn(i) = quad(F_12,x_low,x_high)/nnot(i)* 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   ;  % Why 0.4 here?
    % Use some special function
  end
 disp(['    acid(', num2str(i), ')= ', num2str( acid(i), '%15.5f') , '  taulnew(', num2str(i), ')= ',
num2str( taulnew(i), ...
    '%15.5f') , '   robs = ',  num2str( robs, '%15.5f')]);



 %==================================
 %%%  Reactor 2       %%%
 %==================================
 i=2;
 nnottoler = nnot(i)/500;
 while abs(taulnew(i) - taul(i)) > liqtoler ;
    ndone = 0;
    while ndone < 0.50
       nhat0=nhatzero(i);
       options = odeset('RelTol',1e-3,'AbsTol',1e-3);
        [x,nhat] = ode15s(@chan2,[x_low,x_high],nhat0,options);
       x_2=x;nhat_2=nhat;

       F_2 = @(x_1)interp1(x,nhat,x_1) ;
       nhattot=quad(F_2,x_low,x_high) ;
       disp(['   nhatzero=  ', num2str( nhatzero(i), '%15.5f')  , ' ;    nhattot=  ', ...
           num2str( nhattot, '%15.5f'), ' ;   nnot(', num2str(i) , ')=  ', num2str( nnot(i), '%15.5f') ]);

       if abs(nhattot - nnot(i)) < nnottoler ;
          ndone=1 ;
       end


       if  (nhatzero(i) + (nnot(i) - nhattot) *1.0) > 0
            nhatzero(i)=  nhatzero(i)  + (nnot(i) - nhattot)*0.7;
       else
           nhatzero(i)=  nhatzero(i)  + (nnot(i) - nhattot)*0.7;
       end
     end

    F_22 = @(x_1) interp1(x,nhat,x_1).*x_1 ;
    Convrsn(i) = quad(F_22,x_low,x_high)/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;
      disp([' taulnew(', num2str(i), ')=', num2str(taulnew(i), '%15.5f'),' taul(', num2str(i), ')=',
num2str(taul(i), '%15.5f'), ]);

   end

   disp([' acid(', num2str(i), ')= ', num2str( acid(i), '%15.5f') , ' taulnew(', num2str(i), ')= ',
num2str( taulnew(i), ...
     '%15.5f') , ' robs = ', num2str( robs, '%15.5f')]);

   %==================================
   %%% Reactor 3      %%%
   %==================================
   i=3;
   nnottoler = nnot(i)/500;
   while abs(taulnew(i) - taul(i)) > liqtoler ;
      ndone = 0;
      while ndone < 0.50
         nhat0=nhatzero(i);
         options = odeset('RelTol',1e-3,'AbsTol',1e-3);
          [x,nhat] = ode15s(@chan3,[x_low,x_high],nhat0,options);
         x_3=x;nhat_3=nhat;
         F_3 = @(x_1)interp1(x,nhat,x_1) ;
         nhattot=quad(F_3,x_low,x_high) ;
         disp([' nhatzero= ', num2str( nhatzero(i), '%15.5f') , ' ; nhattot= ', ...
            num2str( nhattot, '%15.5f'), ' ; nnot(', num2str(i) , ')= ', num2str( nnot(i), '%15.5f') ]);

         if abs(nhattot - nnot(i)) < nnottoler ;
            ndone=1 ;
         end


         if (nhatzero(i) + (nnot(i) - nhattot) *1.0) > 0
            nhatzero(i)= nhatzero(i) + (nnot(i) - nhattot)*0.7;
         else
            nhatzero(i)= nhatzero(i) + (nnot(i) - nhattot)*0.7;
         end
      end

      F_32 = @(x_1) interp1(x,nhat,x_1).*x_1 ;
      Convrsn(i) = quad(F_32,x_low,x_high)/nnot(i);
      robs = solidfeed*so/vol(i) *(Convrsn(i) - Convrsn(i-1));  % Eq 3-22

      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;
         disp([' taulnew(', num2str(i), ')=', num2str(taulnew(i), '%15.5f'),' taul(', num2str(i), ')=',
num2str(taul(i), '%15.5f'), ]);
```

```
    end

    disp(['    acid(', num2str(i), ')= ', num2str( acid(i), '%15.5f') , '  taulnew(', num2str(i), ')= ',
num2str( taulnew(i), ...
       '%15.5f') , '   robs = ',  num2str( robs, '%15.5f')]);

    %==================================
    %%%  Reactor 4       %%%
    %==================================

    i = 4;
    nnottoler = nnot(i)/500;
    while abs(taulnew(i) - taul(i)) > liqtoler ;
       ndone = 0;
       while ndone < 0.50
          nhat0=nhatzero(i);
          options = odeset('RelTol',1e-3,'AbsTol',1e-3);
            [x,nhat] = ode15s(@chan4,[x_low,x_high],nhat0,options);
          x_4=x;nhat_4=nhat;

          F_4 = @(x_1)interp1(x,nhat,x_1) ;
          nhattot=quad(F_4,x_low,x_high) ;
          disp(['   nhatzero=  ', num2str( nhatzero(i), '%15.5f')  , ' ;    nhattot=  ', ...
              num2str( nhattot, '%15.5f'), ' ;   nnot(', num2str(i) , ')=  ', num2str( nnot(i), '%15.5f') ]);

          if abs(nhattot - nnot(i)) < nnottoler ;
             ndone=1 ;
          end


          if  (nhatzero(i) + (nnot(i) - nhattot) *1.0) > 0
               nhatzero(i)=  nhatzero(i)  + (nnot(i) - nhattot)*0.7;
          else
               nhatzero(i)=  nhatzero(i)  + (nnot(i) - nhattot)*0.7;
          end

       end

       F_42 = @(x_1) interp1(x,nhat,x_1).*x_1 ;
       Convrsn(i) = quad(F_42,x_low,x_high)/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;
        disp(['   taulnew(', num2str(i), ')=',  num2str(taulnew(i), '%15.5f'),'   taul(', num2str(i), ')=',
num2str(taul(i), '%15.5f'), ]);


    end
```

```
   disp(['    acid(', num2str(i), ')= ', num2str( acid(i), '%15.5f') , '  taulnew(', num2str(i), ')= ',
num2str( taulnew(i), ...
      '%15.5f') , '   robs = ',  num2str( robs, '%15.5f')]);

   disp(['  Conversion in each stage (from nhat):    ', num2str( Convrsn', '%13.5f')]);

   if max(abs(acid-acidold)) < acidtoler
        done=1 ;
   end
   acidold = acid ;

end

%% ========================
%% Output results section
%% ========================
disp('Congratulation! The simulation process is successfully finished!')
toc      % toc is used to check the whole time processed.


for i3=1:(stages+1);
      disp(['    L(', int2str(i3), ')= ', num2str(L(i3))]) ;
end

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);
% Calculation of Destruction
destruction(1) = solidfeed/1000* (Convrsn(1) - 0);
for i3=2:stages;
   destruction(i3)=solidfeed/1000*(Convrsn(i3)-Convrsn(i3-1)) ;
end
selectivi=creation./destruction;
selec = L(1)*acid(1)/(solidfeed *Convrsn(4));

% output the result and plot the result
disp(['    SELECTIVITY =',  num2str(selectivi','%15.5f')]) ;
disp(['    Creation = ',  num2str(creation','%15.5f')]) ;
disp(['    destruction =',  num2str(destruction','%15.5f')]) ;
disp(['    selectivity = ',  num2str(selec,'%15.5f')]) ;
disp(['    tauloverall= ',  num2str(tauloverall,'%15.5f')]) ;
disp(['    taus  = ',  num2str(sum(taus),'%15.5f')]) ;
disp(['    acid levels   = ',  num2str(acid', '%13.5f')]) ;


disp(['    VSLR_LOOP = ',  num2str(VSLR_loop), '    LRT_loop = ',  num2str(LRT_loop)]) ;

% Collect data for CPDM map.
ACID=[ACID;acid(1)];
CONVERSION=[CONVERSION;Convrsn(4)];
```

```
LRT_loop = LRT_loop + 0.5;
end
VSLR_loop = VSLR_loop + 0.5;
end

diary off;  % End of log

% ================================================================
% Section to draw CPDM "map" of product concentration and conversion.
% tested and proved working on 11/15/2004
% ================================================================

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;
VSLR_sort1 = VSLR_sort(uniqueM);
VSLR_number = diff(find([1;uniqueM]));

LRT_sort = sort(mapdata(:,2));
uniqueM = [diff(LRT_sort);1] > 0;
LRT_sort1 = LRT_sort(uniqueM);  %Uniqyue 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);
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;
axis([0 1 0 60]);
% ------ end of Map Ploting

% Open the diary file to print or edition.
YESNO='';
while isempty(YESNO)
   YESNO = input('Do you want to check results from the diary file? Y/N [Y]: ','s');
end

if strcmpi(YESNO , 'Y') == 1
    edit  num2str(resultfile) ;
end
```

******End of the main MATLAB code**
******The following are four function files (i.e., Chan1.m, Chan2.m, Chan3.m, and Chan4.m) used in this main source code.

***Chan1.m

```
function dnhat = nhateq1(x,nhat1)
global so taus a1 b1 c1 d1 e1 f1 i
global ratio acid

rmodel = @(x1,acd) a1.*(1-x1).^b1./(1+c1.*(10.*x1).^d1+e1.*(acd.*ratio).^f1);
drmodel = @(x1,acd) -28341/100000.*(1-
x1)^(101/100)./(1+517/500*3^(273/1000)*5^(727/1000).*acd^(273/1000));

i=1;
dnhatdt =  -nhat1*(drmodel(x,acid(i))+so./taus(i))/rmodel(x,acid(i))  ;
dnhat = [dnhatdt];
```

***Chan2.m

```
function dnhat = nhateq2(x,nhat1)
global so taus a1 b1 c1 d1 e1 f1 i RN
global ratio acid nnot factr1
global x_1 nhat_1 x_2 nhat_2 x_3 nhat_3 x_4 nhat_4


rmodel = @(x1,acd) a1.*(1-x1).^b1./(1+c1.*(10.*x1).^d1+e1.*(acd.*ratio).^f1);
drmodel = @(x1,acd) -28341/100000.*(1-
x1)^(101/100)./(1+517/500*3^(273/1000)*5^(727/1000).*acd^(273/1000));

F_1m = @(x_m)interp1(x_1,nhat_1,x_m) ;
```

```
i=2;
dnhatdt = -nhat1*(drmodel(x,acid(i))+so./taus(i))/rmodel(x,acid(i))+ ...
        F_1m(x).*nnot(i)./nnot(i-1)*factr1*so./taus(i)/rmodel(x,acid(i)) ;

dnhat = [dnhatdt];
```

***Chan3.m

```
function dnhat = chan3(x,nhat1)
global so taus a1 b1 c1 d1 e1 f1 i RN
global ratio acid nnot factr1
global x_1 nhat_1 x_2 nhat_2 x_3 nhat_3 x_4 nhat_4

rmodel = @(x1,acd) a1.*(1-x1).^b1./(1+c1.*(10.*x1).^d1+e1.*(acd.*ratio).^f1);
drmodel = @(x1,acd) -2247/5000*(1-
x1)^(271/50)/(1+6741/31250*21^(33/100)*25^(67/100)*acd^(133/100));
F_2m = @(x_m)interp1(x_2,nhat_2,x_m) ;

i=3;
dnhatdt = -nhat1*(drmodel(x,acid(i))+so./taus(i))/rmodel(x,acid(i))+ ...
        F_2m(x).*nnot(i)./nnot(i-1)*so./taus(i)/rmodel(x,acid(i)) ;

dnhat = [dnhatdt];
```

***Chan4.m

```
function dnhat = nhateq4(x,nhat1)
global so taus a1 b1 c1 d1 e1 f1 i RN
global ratio acid nnot factr1
global x_1 nhat_1 x_2 nhat_2 x_3 nhat_3 x_4 nhat_4


rmodel = @(x1,acd) a1.*(1-x1).^b1./(1+c1.*(10.*x1).^d1+e1.*(acd.*ratio).^f1);
drmodel = @(x1,acd) -28341/100000.*(1-
x1)^(101/100)./(1+517/500*3^(273/1000)*5^(727/1000).*acd^(273/1000));
F_3m = @(x_m)interp1(x_3,nhat_3,x_m) ;

i=4;
dnhatdt = -nhat1*(drmodel(x,acid(i))+so./taus(i))/rmodel(x,acid(i))+ ...
        F_3m(x).*nnot(i)./nnot(i-1)*so./taus(i)/rmodel(x,acid(i)) ;

dnhat = [dnhatdt];
```

APPENDIX J

MATLAB CODE FOR CPDM PREDICTION MAP

```
% ========================================================================
%   Conversion and acid concentration "map" for CPDM Method.
% - This source code can be used standalone or combined in the MATLAB codes (Appendix I).
% - Program is used to predict acid concentration and conversion for a range of VSLRs and LRTs.
% -This code was made and tested by Zhihong Fu on 10/05/2004
%  Department of Chemical Engineering, Texas A&M University, College Station, TX
% ========================================================================


clear all
close all
global so taus a1 b1 c1 d1 e1 f1
global holdup moist ratio stages loading tauloverall
global ratio acid nnot factr1
global x_1 nhat_1 x_2 nhat_2 x_3 nhat_3 x_4 nhat_4


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);  %Uniqyue 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;
axis([0 1 0 60]);
```

APPENDIX K

PERL SCRIPT TO CONVERT GC DATA

This perl script code was used to produce the formula for EXCEL file and automatically convert the duplicate carboxylic acid concentration from GC original EXCEL output to the average carboxylic acids concentration which can be further converted to Aceq.

```
#open output text file;
open (LOGFILE, '> CPDM.txt') ;
print LOGFILE  "DAY  C2 (g/L)          C3 (g/L)          IC4 (g/L)          C4 (g/L)          IC5
(g/L)     C5 (g/L)          C6 (g/L)          C7 (g/L)          Total (g/L)\n";
@label = split(/ +/, "A B C D E F G H I J K L M N O P Q R S T U V W X Y Z ");
for ($count=1; $count<500; $count++)
{
        my $tempcount = $count+1;
        my $output = ();
        foreach my $letter (split/\s +/, "A B C D E F G H I J K L M N O P Q R S T U V W X Y
Z ") {
                $output .=  "=AVERAGE($letter$count:$letter$tempcount)        ";
        }
        $output =~ s/     $//;
        $output .= "\n";
        #print LOGFILE  "=AVERAGE(C$count:C$tempcount)
        =AVERAGE(D$count:D$tempcount)     =AVERAGE(E$count:E$tempcount)
        =AVERAGE(F$count:F$tempcount)     =AVERAGE(G$count:G$tempcount)
        =AVERAGE(H$count:H$tempcount)     =AVERAGE(I$count:I$tempcount)
        =AVERAGE(J$count:J$tempcount)     =AVERAGE(K$count:K$tempcount)
        =AVERAGE(L$count:L$tempcount)\n" ;
        print LOGFILE $output;
        $count++
}

close LOGFILE;
```

APPENDIX L
CARBOXYLIC ACID PRODUCTION DATA FOR COMPARISON OF LAKE INOCULUM AND MARINE INOCULUM

Table L-1. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse Fermentation MS1 (original "black" lake inocula, ammonium bicarbonate buffer, and 55°C).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	2.291	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.291
2	3.502	0.105	0.000	0.697	0.000	0.000	0.000	0.000	4.304
4	5.364	0.191	0.000	0.866	0.000	0.000	0.000	0.000	6.422
6	7.156	0.226	0.000	1.052	0.000	0.000	0.000	0.000	8.435
8	8.321	0.208	0.063	1.293	0.053	0.000	0.000	0.000	9.938
10	9.693	0.203	0.000	1.520	0.082	0.000	0.000	0.000	11.497
12	10.047	0.243	0.110	1.613	0.119	0.000	0.000	0.000	12.132
14	10.796	0.221	0.128	1.785	0.129	0.000	0.000	0.000	13.059
16	11.020	0.256	0.156	1.891	0.158	0.000	0.000	0.000	13.48
18	11.315	0.274	0.167	1.886	0.185	0.000	0.000	0.050	13.878
20	11.927	0.277	0.188	1.909	0.215	0.000	0.000	0.000	14.517
22	12.825	0.197	0.210	1.975	0.250	0.000	0.000	0.000	15.458
24	13.025	0.138	0.232	1.991	0.267	0.000	0.000	0.000	15.652
26	13.362	0.148	0.249	2.024	0.286	0.000	0.000	0.000	16.069
28	13.215	0.116	0.261	2.027	0.282	0.000	0.000	0.059	15.960
30	12.942	0.116	0.267	2.030	0.280	0.000	0.000	0.078	15.712
32	13.732	0.000	0.276	2.202	0.288	0.000	0.000	0.000	16.498
38	17.813	0.192	0.227	1.954	0.314	0.000	0.000	0.094	20.593
40	18.715	0.163	0.255	2.077	0.353	0.000	0.000	0.132	21.695
42	16.942	0.137	0.240	1.936	0.341	0.000	0.000	0.145	19.741
46	16.608	0.149	0.201	1.869	0.375	0.000	0.000	0.000	19.203
49	15.983	0.159	0.159	1.700	0.400	0.000	0.000	0.000	18.401

Table L-2. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse Fermentation MS2 (original "black" lake inocula, ammonium bicarbonate buffer, and 55°C).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	2.200	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.2
2	3.252	0.102	0.000	0.805	0.000	0.000	0.000	0.000	4.159
4	5.203	0.169	0.000	0.889	0.000	0.000	0.000	0.000	6.262
6	7.241	0.252	0.000	1.178	0.000	0.000	0.000	0.000	8.671
8	8.099	0.191	0.072	1.316	0.057	0.000	0.000	0.000	9.735
10	9.082	0.173	0.089	1.469	0.080	0.000	0.000	0.000	10.892
12	10.163	0.241	0.122	1.565	0.125	0.000	0.000	0.000	12.217
14	11.593	0.252	0.000	1.638	0.149	0.000	0.000	0.000	13.632
16	11.800	0.305	0.165	1.756	0.174	0.000	0.000	0.000	14.2
18	12.564	0.338	0.181	1.770	0.206	0.000	0.000	0.000	15.061
20	13.040	0.312	0.204	1.818	0.242	0.000	0.000	0.000	15.616
22	14.146	0.246	0.229	1.911	0.278	0.000	0.000	0.000	16.81
24	13.721	0.146	0.244	1.894	0.281	0.000	0.000	0.000	16.287
26	13.828	0.140	0.000	1.905	0.275	0.000	0.000	0.000	16.148
28	14.181	0.138	0.255	1.922	0.272	0.000	0.000	0.000	16.769
30	13.523	0.120	0.000	1.897	0.284	0.000	0.000	0.000	15.823
32	13.999	0.110	0.204	1.943	0.309	0.000	0.000	0.049	16.614
38	17.844	0.197	0.158	1.736	0.348	0.000	0.000	0.000	20.284
40	19.264	0.165	0.167	1.879	0.374	0.000	0.000	0.078	21.927
42	17.576	0.145	0.145	1.778	0.357	0.000	0.000	0.000	20.001
46	18.119	0.168	0.142	1.844	0.394	0.000	0.000	0.000	20.665
49	17.852	0.175	0.123	1.724	0.417	0.000	0.000	0.000	20.292

Table L-3. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse Fermentation MS3 (original "brown" lake inocula, ammonium bicarbonate buffer, and 55°C).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	2.529	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.529
2	3.948	0.118	0.000	0.767	0.000	0.000	0.000	0.000	4.832
4	5.556	0.185	0.000	1.016	0.000	0.000	0.000	0.000	6.757
6	7.788	0.256	0.000	1.419	0.071	0.000	0.000	0.000	9.534
8	8.917	0.225	0.081	1.650	0.097	0.000	0.000	0.000	10.971
10	10.254	0.202	0.102	1.812	0.123	0.000	0.050	0.000	12.543
12	11.604	0.234	0.146	2.002	0.168	0.000	0.049	0.000	14.203
14	12.319	0.238	0.168	2.103	0.179	0.000	0.050	0.000	15.056
16	12.495	0.278	0.191	2.263	0.204	0.000	0.055	0.000	15.485
18	14.031	0.325	0.214	2.411	0.241	0.000	0.000	0.000	17.222
20	15.270	0.328	0.235	2.476	0.268	0.000	0.000	0.000	18.576
22	16.207	0.267	0.000	2.435	0.276	0.000	0.055	0.000	19.241
24	17.627	0.227	0.271	2.530	0.286	0.000	0.000	0.000	20.942
26	18.862	0.224	0.264	2.513	0.270	0.000	0.050	0.000	22.182
28	18.862	0.200	0.251	2.516	0.263	0.000	0.000	0.046	22.138
30	19.078	0.202	0.235	2.496	0.281	0.000	0.000	0.060	22.352
32	20.107	0.184	0.219	2.595	0.301	0.000	0.000	0.064	23.47
38	22.247	0.247	0.201	2.441	0.349	0.000	0.000	0.088	25.572
40	21.644	0.205	0.218	2.414	0.368	0.000	0.000	0.106	24.955
42	19.421	0.173	0.206	2.300	0.361	0.000	0.000	0.115	22.577
46	19.301	0.195	0.182	2.267	0.390	0.000	0.000	0.143	22.479
49	18.236	0.177	0.000	2.092	0.400	0.000	0.000	0.159	21.063

Table L-4. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse Fermentation MS4 (original "brown" lake inocula, ammonium bicarbonate buffer, and 55°C).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	2.101	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.101
2	3.789	0.087	0.000	0.542	0.000	0.000	0.000	0.000	4.419
4	5.609	0.111	0.000	0.715	0.000	0.000	0.000	0.000	6.434
6	8.165	0.227	0.064	0.985	0.062	0.000	0.000	0.000	9.503
8	9.025	0.220	0.088	1.249	0.092	0.000	0.000	0.000	10.673
10	9.586	0.174	0.099	1.413	0.107	0.000	0.000	0.000	11.379
12	9.407	0.229	0.128	1.698	0.135	0.000	0.000	0.000	11.597
14	9.474	0.228	0.000	1.781	0.145	0.000	0.000	0.000	11.628
16	8.980	0.249	0.150	1.840	0.163	0.000	0.000	0.000	11.381
18	10.062	0.246	0.137	1.819	0.161	0.000	0.000	0.000	12.424
20	11.392	0.229	0.143	1.820	0.171	0.000	0.051	0.000	13.806
22	12.992	0.193	0.156	1.956	0.187	0.000	0.054	0.000	15.538
24	13.290	0.155	0.167	2.007	0.196	0.000	0.054	0.000	15.868
26	15.310	0.176	0.000	2.073	0.201	0.000	0.055	0.000	17.816
28	16.552	0.172	0.182	2.187	0.205	0.000	0.000	0.000	19.298
30	17.387	0.154	0.000	2.263	0.205	0.000	0.053	0.073	20.136
32	18.088	0.130	0.188	2.388	0.205	0.000	0.056	0.087	21.142
38	19.292	0.204	0.175	2.262	0.249	0.000	0.000	0.099	22.282
40	19.050	0.181	0.178	2.318	0.268	0.000	0.000	0.113	22.108
42	17.127	0.157	0.172	2.155	0.255	0.000	0.000	0.125	19.991
46	17.197	0.182	0.165	2.178	0.278	0.000	0.000	0.146	20.145
49	16.845	0.170	0.144	2.073	0.289	0.000	0.000	0.162	19.683

Table L-5. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse Fermentation MS5 (mixture of 50% of original "brown" lake inocula and 50% of original "black" inoculum, ammonium bicarbonate buffer, and 55°C).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	2.354	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.354
2	3.672	0.109	0.000	0.689	0.000	0.000	0.000	0.000	4.47
4	5.414	0.132	0.000	0.821	0.000	0.000	0.000	0.000	6.368
6	8.204	0.237	0.000	1.297	0.056	0.000	0.000	0.000	9.795
8	9.332	0.252	0.000	1.629	0.096	0.000	0.000	0.000	11.309
10	10.238	0.219	0.103	1.775	0.131	0.000	0.000	0.000	12.466
12	10.999	0.278	0.156	1.997	0.192	0.000	0.000	0.000	13.622
14	11.972	0.266	0.000	2.205	0.219	0.000	0.000	0.000	14.661
16	11.688	0.302	0.222	2.298	0.247	0.000	0.000	0.000	14.758
18	11.487	0.321	0.234	2.312	0.270	0.000	0.000	0.000	14.624
20	12.144	0.328	0.267	2.403	0.317	0.000	0.000	0.000	15.459
22	13.215	0.284	0.000	2.498	0.346	0.000	0.000	0.000	16.344
24	13.145	0.204	0.300	2.496	0.343	0.000	0.000	0.000	16.488
26	13.987	0.195	0.309	2.502	0.329	0.000	0.000	0.000	17.322
28	14.325	0.176	0.297	2.486	0.305	0.000	0.000	0.000	17.589
30	13.812	0.151	0.262	2.447	0.313	0.000	0.000	0.050	17.036
32	14.745	0.000	0.241	2.554	0.348	0.000	0.000	0.000	17.888
38	21.352	0.235	0.189	2.414	0.364	0.000	0.000	0.087	24.641
40	20.610	0.203	0.208	2.420	0.402	0.000	0.000	0.098	23.94
42	17.949	0.165	0.215	2.278	0.402	0.000	0.000	0.109	21.118
46	17.703	0.190	0.211	2.299	0.425	0.000	0.000	0.126	20.953
49	17.064	0.170	0.191	2.187	0.442	0.000	0.000	0.145	20.2

Table L-6. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse Fermentation MS6 (mixture of 50% of original "brown" lake inocula and 50% of original "black" inoculum, ammonium bicarbonate buffer, and 55°C).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	2.526	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.526
2	3.865	0.123	0.000	1.029	0.000	0.000	0.000	0.000	5.017
4	6.705	0.214	0.000	1.247	0.000	0.000	0.000	0.000	8.165
6	8.827	0.275	0.078	1.582	0.075	0.000	0.000	0.000	10.838
8	9.943	0.265	0.111	1.914	0.113	0.000	0.000	0.000	12.346
10	10.650	0.205	0.130	2.012	0.141	0.000	0.000	0.000	13.139
12	10.809	0.240	0.168	2.153	0.186	0.000	0.000	0.000	13.557
14	11.467	0.245	0.000	2.316	0.208	0.000	0.000	0.000	14.236
16	11.452	0.277	0.213	2.494	0.222	0.000	0.000	0.000	14.657
18	12.060	0.307	0.214	2.479	0.234	0.000	0.000	0.065	15.359
20	13.978	0.308	0.221	2.493	0.248	0.000	0.000	0.000	17.248
22	15.395	0.289	0.240	2.600	0.275	0.000	0.000	0.000	18.799
24	15.786	0.234	0.256	2.630	0.281	0.000	0.000	0.000	19.187
26	16.250	0.234	0.258	2.617	0.272	0.000	0.000	0.055	19.685
28	17.039	0.209	0.253	2.667	0.261	0.000	0.000	0.000	20.429
30	16.048	0.186	0.220	2.543	0.268	0.000	0.000	0.000	19.265
32	17.124	0.160	0.000	2.650	0.295	0.000	0.000	0.000	20.229
38	23.420	0.253	0.186	2.562	0.336	0.000	0.000	0.053	26.811
40	22.675	0.199	0.177	2.538	0.348	0.000	0.000	0.060	25.996
42	19.988	0.172	0.189	2.412	0.353	0.000	0.000	0.076	23.189
46	19.698	0.188	0.203	2.485	0.388	0.000	0.000	0.090	23.053
49	19.035	0.185	0.186	2.389	0.400	0.000	0.000	0.101	22.297

Table L-7. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse Fermentation MS7 (original marine inocula, ammonium bicarbonate buffer, and 55°C).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	2.397	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.397
2	3.864	0.000	0.000	0.381	0.000	0.000	0.000	0.000	4.246
4	6.547	0.156	0.000	0.620	0.000	0.000	0.000	0.000	7.323
6	9.129	0.242	0.081	1.005	0.068	0.000	0.000	0.000	10.524
8	10.339	0.241	0.102	1.228	0.099	0.000	0.000	0.000	12.01
10	11.163	0.197	0.116	1.453	0.127	0.000	0.000	0.000	13.056
12	11.645	0.249	0.126	1.617	0.173	0.000	0.000	0.000	13.81
14	12.099	0.259	0.000	1.816	0.222	0.000	0.000	0.000	14.395
16	11.111	0.290	0.182	1.883	0.254	0.000	0.000	0.000	13.72
18	11.525	0.277	0.188	1.903	0.269	0.000	0.000	0.000	14.162
20	13.291	0.263	0.203	1.936	0.293	0.000	0.000	0.000	15.985
22	15.326	0.205	0.222	2.040	0.317	0.000	0.000	0.000	18.11
24	15.111	0.171	0.221	2.063	0.313	0.000	0.000	0.046	17.925
26	16.531	0.186	0.233	2.118	0.335	0.000	0.000	0.000	19.403
28	16.485	0.171	0.235	2.142	0.317	0.000	0.000	0.000	19.35
30	17.029	0.176	0.238	2.244	0.307	0.000	0.000	0.081	20.074
32	17.960	0.170	0.256	2.384	0.308	0.000	0.000	0.000	21.078
38	21.746	0.237	0.251	2.400	0.331	0.000	0.000	0.103	25.067
40	21.330	0.212	0.272	2.447	0.370	0.000	0.000	0.119	24.749
42	18.776	0.185	0.268	2.319	0.360	0.000	0.000	0.131	22.038
46	18.756	0.220	0.273	2.383	0.376	0.000	0.000	0.154	22.162
49	18.379	0.211	0.255	2.322	0.381	0.000	0.000	0.169	21.717

Table L-8. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse Fermentation MS9 (adapted marine inocula from previous ammonium bicarbonate countercurrent fermentations, ammonium bicarbonate buffer, and 55°C).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	5.113	0.078	0.000	0.346	0.000	0.000	0.000	0.000	5.536
2	6.757	0.096	0.000	1.254	0.000	0.000	0.000	0.000	8.106
4	8.460	0.152	0.066	1.389	0.000	0.000	0.000	0.000	10.068
6	11.155	0.300	0.000	1.646	0.000	0.000	0.000	0.000	13.101
8	11.830	0.272	0.101	1.801	0.077	0.000	0.000	0.000	14.081
10	12.596	0.233	0.117	2.040	0.104	0.000	0.000	0.000	15.09
12	13.423	0.298	0.136	2.264	0.144	0.000	0.000	0.000	16.266
14	14.080	0.282	0.154	2.374	0.175	0.000	0.000	0.048	17.113
16	13.138	0.277	0.175	2.420	0.206	0.000	0.000	0.074	16.29
18	13.423	0.307	0.187	2.474	0.232	0.000	0.000	0.000	16.622
20	14.781	0.309	0.214	2.585	0.265	0.000	0.000	0.068	18.222
22	16.195	0.272	0.230	2.731	0.290	0.000	0.000	0.059	19.777
24	16.323	0.215	0.246	2.754	0.309	0.000	0.000	0.065	19.912
26	18.123	0.246	0.265	2.794	0.320	0.000	0.000	0.143	21.892
28	19.192	0.256	0.275	2.902	0.319	0.000	0.000	0.074	23.017
30	18.577	0.236	0.263	2.875	0.288	0.000	0.000	0.080	22.317
32	19.585	0.201	0.268	3.012	0.276	0.000	0.000	0.092	23.433
38	25.866	0.290	0.250	2.991	0.318	0.000	0.000	0.113	29.828
40	24.613	0.252	0.000	3.038	0.370	0.000	0.000	0.123	28.396
42	22.212	0.225	0.277	2.900	0.368	0.000	0.000	0.135	26.116
46	22.383	0.270	0.000	3.000	0.382	0.000	0.000	0.149	26.185
49	21.758	0.241	0.263	2.907	0.379	0.000	0.000	0.161	25.71

Table L-9. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse Fermentation MS10 (adapted marine inocula from previous ammonium bicarbonate countercurrent thermophilic fermentations, ammonium bicarbonate buffer, and 55°C).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	5.326	0.084	0.000	0.356	0.000	0.000	0.000	0.000	5.766
2	6.641	0.127	0.000	1.514	0.000	0.000	0.000	0.000	8.282
4	8.899	0.122	0.000	1.596	0.000	0.000	0.000	0.000	10.617
6	11.086	0.227	0.000	1.894	0.000	0.000	0.000	0.000	13.207
8	11.818	0.231	0.103	2.073	0.091	0.000	0.000	0.000	14.315
10	12.108	0.199	0.110	2.148	0.103	0.000	0.000	0.000	14.668
12	12.441	0.234	0.126	2.231	0.128	0.000	0.000	0.000	15.161
14	13.239	0.235	0.000	2.351	0.000	0.000	0.000	0.050	15.874
16	13.265	0.000	0.000	2.374	0.000	0.000	0.000	0.000	15.639
18	14.484	0.286	0.177	2.452	0.201	0.000	0.000	0.000	17.6
20	15.149	0.275	0.185	2.399	0.214	0.000	0.000	0.113	18.335
22	17.040	0.263	0.207	2.545	0.244	0.000	0.000	0.127	20.426
24	16.901	0.229	0.218	2.577	0.257	0.000	0.000	0.122	20.303
26	18.226	0.252	0.227	2.711	0.265	0.000	0.209	0.000	21.89
28	18.831	0.233	0.231	2.758	0.267	0.000	0.000	0.143	22.463
30	18.023	0.215	0.219	2.731	0.239	0.000	0.000	0.154	21.58
32	18.968	0.229	0.219	2.835	0.244	0.000	0.000	0.165	22.659
38	24.893	0.306	0.183	2.923	0.279	0.000	0.000	0.158	28.742
40	24.014	0.250	0.000	2.969	0.307	0.000	0.000	0.165	27.705
42	22.085	0.213	0.212	2.847	0.314	0.000	0.000	0.168	25.839
46	21.857	0.243	0.215	2.892	0.332	0.000	0.000	0.170	25.709
49	21.762	0.239	0.203	2.814	0.345	0.000	0.000	0.183	25.546

Table L-10. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse Fermentation CS1 (original "black" lake inocula, ammonium bicarbonate buffer, and 40°C).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	2.176	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.176
2	2.954	0.075	0.000	0.451	0.000	0.000	0.000	0.000	3.481
4	5.753	0.073	0.000	0.620	0.000	0.000	0.000	0.000	6.445
6	8.875	0.231	0.064	0.812	0.000	0.000	0.000	0.000	9.982
8	10.589	0.482	0.132	1.182	0.072	0.000	0.000	0.000	12.456
10	12.020	0.455	0.157	1.418	0.097	0.000	0.000	0.000	14.146
12	12.537	0.281	0.183	1.595	0.127	0.000	0.000	0.000	14.724
14	13.267	0.202	0.000	1.689	0.158	0.000	0.000	0.134	15.45
16	12.689	0.179	0.242	1.778	0.205	0.000	0.000	0.000	15.093
18	12.529	0.162	0.271	1.878	0.251	0.000	0.000	0.000	15.092
20	12.344	0.145	0.296	1.955	0.288	0.000	0.000	0.046	15.074
22	13.123	0.156	0.335	2.081	0.345	0.000	0.000	0.000	16.039
24	12.984	0.111	0.361	2.125	0.382	0.000	0.000	0.000	15.962
26	12.673	0.095	0.384	2.093	0.405	0.000	0.000	0.000	15.65
28	13.372	0.099	0.424	2.214	0.462	0.000	0.000	0.000	16.572
30	12.326	0.094	0.434	2.156	0.487	0.000	0.000	0.000	15.498
32	12.884	0.089	0.497	2.254	0.549	0.000	0.000	0.000	16.273
38	13.074	0.146	0.501	2.120	0.605	0.000	0.000	0.090	16.536
40	12.562	0.082	0.526	2.142	0.645	0.000	0.000	0.000	15.956
42	10.343	0.000	0.534	1.987	0.630	0.000	0.000	0.000	13.493
46	10.802	0.085	0.594	2.055	0.715	0.000	0.000	0.000	14.251
49	8.979	0.000	0.628	1.543	0.694	0.000	0.000	0.153	11.996

Table L-11. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse Fermentation CS2 (original "black" lake inocula, ammonium bicarbonate buffer, and 40°C).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	2.196	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.196
2	3.280	0.086	0.000	0.582	0.000	0.000	0.000	0.000	3.948
4	5.329	0.000	0.000	0.818	0.000	0.000	0.000	0.000	6.148
6	8.683	0.849	0.000	0.798	0.000	0.000	0.000	0.000	10.33
8	10.851	1.246	0.076	1.002	0.055	0.000	0.000	0.000	13.231
10	11.830	1.233	0.107	1.105	0.103	0.000	0.000	0.000	14.379
12	13.075	1.139	0.149	1.253	0.161	0.000	0.000	0.000	15.777
14	13.614	0.859	0.170	1.276	0.187	0.000	0.000	0.000	16.106
16	13.416	0.645	0.190	1.315	0.215	0.000	0.000	0.000	15.782
18	13.862	0.409	0.214	1.354	0.244	0.000	0.000	0.000	16.082
20	14.969	0.273	0.248	1.434	0.286	0.000	0.000	0.000	17.209
22	15.537	0.233	0.268	1.466	0.302	0.000	0.000	0.000	17.806
24	15.899	0.162	0.000	1.494	0.331	0.000	0.000	0.000	17.886
26	15.491	0.119	0.308	1.443	0.337	0.000	0.000	0.099	17.798
28	15.479	0.092	0.329	1.424	0.357	0.000	0.000	0.000	17.68
30	14.571	0.088	0.330	1.344	0.362	0.000	0.000	0.000	16.696
32	15.306	0.086	0.380	1.358	0.401	0.000	0.000	0.000	17.53
38	15.011	0.000	0.381	1.233	0.410	0.000	0.000	0.000	17.034
40	15.381	0.096	0.395	1.267	0.445	0.000	0.000	0.000	17.584
42	13.466	0.075	0.406	1.144	0.431	0.000	0.000	0.000	15.523
46	14.417	0.121	0.422	1.131	0.446	0.000	0.000	0.000	16.537
49	13.976	0.107	0.450	1.090	0.464	0.000	0.000	0.113	16.2

Table L-12. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse Fermentation CS3 (original marine inocula, ammonium bicarbonate buffer, and 40°C).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	2.306	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.306
2	2.720	0.000	0.000	0.695	0.000	0.000	0.000	0.000	3.415
4	4.667	0.000	0.000	1.231	0.000	0.000	0.000	0.000	5.897
6	6.787	0.325	0.000	1.476	0.000	0.000	0.000	0.000	8.588
8	7.673	0.634	0.000	1.942	0.000	0.000	0.000	0.000	10.25
10	8.776	0.657	0.083	2.164	0.053	0.000	0.000	0.000	11.733
12	9.112	0.580	0.108	2.152	0.085	0.000	0.000	0.000	12.036
14	9.282	0.446	0.000	2.115	0.101	0.000	0.000	0.000	11.944
16	8.840	0.372	0.137	2.062	0.120	0.000	0.091	0.000	11.623
18	8.881	0.256	0.153	2.048	0.136	0.000	0.152	0.000	11.627
20	8.908	0.239	0.173	2.199	0.157	0.000	0.316	0.052	12.044
22	9.347	0.198	0.201	2.715	0.187	0.069	0.786	0.074	13.578
24	9.012	0.126	0.238	3.228	0.216	0.088	1.483	0.084	14.475
26	9.138	0.117	0.259	3.247	0.246	0.094	1.708	0.104	14.913
28	8.876	0.094	0.280	3.208	0.268	0.097	1.805	0.106	14.734
30	8.476	0.099	0.297	3.109	0.299	0.097	1.819	0.130	14.326
32	9.016	0.000	0.361	3.217	0.361	0.000	1.933	0.154	15.042
38	9.314	0.179	0.374	2.965	0.430	0.096	1.803	0.173	15.334
40	9.177	0.112	0.000	2.856	0.463	0.097	1.837	0.186	14.728
42	8.286	0.082	0.431	2.576	0.468	0.095	1.807	0.205	13.95
46	8.312	0.096	0.448	2.432	0.500	0.094	1.824	0.228	13.934
49	7.943	0.085	0.475	2.204	0.518	0.094	1.819	0.248	13.387

Table L-13. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse Fermentation CS4 (adapted marine inocula from previous ammonium bicarbonate countercurrent thermophilic fermentations, ammonium bicarbonate buffer, and 40°C).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	5.889	0.089	0.000	0.381	0.000	0.000	0.000	0.000	6.359
2	6.291	0.000	0.000	1.141	0.000	0.000	0.000	0.000	7.432
4	8.582	0.000	0.000	1.228	0.000	0.000	0.000	0.000	9.811
6	10.880	0.249	0.000	1.412	0.000	0.000	0.000	0.000	12.541
8	11.976	0.590	0.000	1.663	0.000	0.000	0.000	0.000	14.23
10	12.737	0.704	0.070	2.170	0.000	0.000	0.000	0.000	15.682
12	13.322	0.606	0.094	2.471	0.073	0.000	0.000	0.000	16.566
14	13.766	0.415	0.000	2.528	0.000	0.000	0.000	0.000	16.709
16	13.291	0.296	0.139	2.577	0.117	0.000	0.000	0.057	16.475
18	12.991	0.227	0.170	2.560	0.172	0.000	0.000	0.063	16.185
20	13.291	0.214	0.218	2.723	0.249	0.000	0.000	0.070	16.765
22	14.872	0.233	0.266	2.924	0.329	0.000	0.000	0.088	18.711
24	14.764	0.163	0.291	3.004	0.367	0.000	0.000	0.093	18.682
26	14.534	0.138	0.315	2.940	0.399	0.000	0.000	0.057	18.383
28	14.096	0.116	0.336	2.995	0.435	0.000	0.000	0.104	18.083
30	13.230	0.000	0.354	2.955	0.467	0.000	0.000	0.000	17.005
32	13.611	0.098	0.399	3.091	0.523	0.000	0.000	0.133	17.856
38	14.474	0.142	0.421	3.057	0.588	0.000	0.000	0.142	18.823
40	14.019	0.106	0.448	3.042	0.616	0.000	0.000	0.152	18.383
42	12.065	0.081	0.451	2.768	0.584	0.000	0.000	0.157	16.106
46	12.252	0.104	0.469	2.801	0.617	0.000	0.000	0.171	16.415
49	11.052	0.078	0.507	2.536	0.636	0.000	0.000	0.181	14.990

Table L-14. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse Fermentation CS5 (original "brown" lake inocula, ammonium bicarbonate buffer, and 40°C).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	2.486	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.486
2	5.158	0.177	0.000	0.736	0.000	0.000	0.000	0.000	6.072
4	8.021	0.383	0.000	0.983	0.000	0.000	0.000	0.000	9.387
6	9.479	0.855	0.086	1.285	0.067	0.000	0.000	0.000	11.771
8	11.617	0.862	0.120	1.586	0.118	0.000	0.000	0.000	14.304
10	12.665	0.730	0.159	1.766	0.174	0.000	0.000	0.000	15.494
12	15.347	0.661	0.000	1.962	0.222	0.000	0.000	0.000	18.192
14	16.857	0.528	0.000	2.150	0.271	0.000	0.096	0.099	20.000
16	17.351	0.346	0.250	2.219	0.297	0.000	0.143	0.118	20.725
18	17.106	0.246	0.271	2.415	0.330	0.000	0.186	0.066	20.619
20	16.456	0.186	0.284	2.546	0.355	0.000	0.209	0.143	20.178
22	17.135	0.183	0.331	2.794	0.412	0.000	0.230	0.101	21.187
28	17.981	0.274	0.368	2.834	0.504	0.000	0.214	0.124	22.299
30	16.535	0.159	0.385	2.731	0.512	0.000	0.222	0.000	20.544
32	15.740	0.121	0.398	2.680	0.524	0.000	0.223	0.153	19.84
36	15.777	0.145	0.417	2.730	0.552	0.000	0.237	0.170	20.028
39	15.360	0.136	0.442	2.674	0.576	0.000	0.235	0.180	19.602

APPENDIX M

CARBOXYLIC ACID PRODUCTION DATA FOR HOT-LIME-WATER-TREATED BAGASSE COUNTERCURRENT FERMENTATIONS BUFFERED BY CALCIUM CARBONATE

Table M-1. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse countercurrent Fermentation CA (marine inocula, calcium carbonate buffer, LRT = 25.85 day, and VSLR = 3.26 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
2	4.002	0.000	0.000	0.963	0.000	0.000	0.000	0.000	4.965
6	4.767	0.000	0.000	2.569	0.000	0.000	0.000	0.000	7.336
8	5.512	0.000	0.000	2.778	0.000	0.000	0.125	0.000	8.415
10	5.782	0.000	0.000	2.919	0.000	0.000	0.180	0.000	8.881
16	7.592	0.299	0.000	3.079	0.000	0.000	0.248	0.000	11.218
23	9.009	0.356	0.000	3.492	0.000	0.000	0.271	0.000	13.129
26	10.700	0.373	0.000	3.794	0.000	0.000	0.290	0.000	15.158
27	10.349	0.312	0.000	3.773	0.000	0.000	0.290	0.000	14.723
31	11.861	0.291	0.000	4.026	0.000	0.000	0.244	0.000	16.423
34	10.739	0.243	0.000	3.988	0.000	0.000	0.220	0.000	15.191
35	12.147	0.265	0.000	4.717	0.000	0.000	0.273	0.000	17.402
41	12.340	0.298	0.000	4.569	0.000	0.000	0.269	0.000	17.475
42	13.030	0.286	0.000	4.547	0.000	0.000	0.260	0.000	18.122
58	17.858	0.406	0.093	4.501	0.068	0.000	0.194	0.000	23.120
60	17.499	0.373	0.000	4.279	0.000	0.000	0.191	0.000	22.342
62	17.383	0.400	0.000	4.189	0.000	0.000	0.147	0.000	22.120
64	17.018	0.406	0.000	4.347	0.000	0.000	0.112	0.000	21.883
66	16.763	0.422	0.000	4.853	0.000	0.000	0.158	0.000	22.197
68	15.990	0.432	0.000	5.337	0.000	0.000	0.263	0.000	22.022
72	12.987	0.393	0.000	5.779	0.000	0.000	0.343	0.000	19.503
74	11.506	0.399	0.000	5.314	0.000	0.000	0.374	0.000	17.593
76	11.416	0.429	0.000	5.584	0.000	0.000	0.447	0.000	17.877
78	10.511	0.355	0.000	5.307	0.000	0.000	0.390	0.000	16.563
80	10.229	0.353	0.000	5.305	0.000	0.000	0.437	0.000	16.325
84	10.765	0.509	0.000	5.610	0.000	0.000	0.470	0.000	17.354
86	10.301	0.449	0.000	5.778	0.000	0.000	0.425	0.000	16.952
88	9.771	0.387	0.000	5.586	0.000	0.000	0.384	0.000	16.127

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
90	9.218	0.464	0.000	5.487	0.000	0.000	0.385	0.000	15.554
92	8.402	0.400	0.000	5.199	0.000	0.000	0.311	0.000	14.312
94	8.193	0.426	0.000	5.908	0.000	0.000	0.298	0.000	14.825
96	7.748	0.374	0.000	5.866	0.000	0.000	0.261	0.000	14.249
98	7.670	0.317	0.000	6.137	0.000	0.000	0.240	0.000	14.364
100	7.322	0.294	0.000	5.857	0.000	0.000	0.239	0.000	13.712
102	7.648	0.339	0.000	5.912	0.000	0.000	0.273	0.000	14.174
104	7.303	0.281	0.000	5.162	0.000	0.000	0.239	0.000	12.984
106	7.437	0.340	0.000	5.911	0.000	0.000	0.278	0.000	13.967
108	8.072	0.333	0.000	5.654	0.000	0.000	0.299	0.000	14.358
110	7.762	0.338	0.000	5.792	0.000	0.000	0.286	0.000	14.177
112	7.460	0.305	0.000	5.599	0.000	0.000	0.256	0.000	13.620
114	7.216	0.307	0.000	5.471	0.000	0.000	0.286	0.000	13.279
116	7.771	0.322	0.000	5.190	0.000	0.000	0.268	0.000	13.551
119	8.467	0.296	0.000	4.538	0.000	0.000	0.248	0.000	13.549
120	9.352	0.327	0.000	4.440	0.000	0.000	0.265	0.000	14.384
122	9.197	0.302	0.000	4.417	0.000	0.000	0.264	0.000	14.180
124	8.436	0.252	0.000	4.197	0.000	0.000	0.226	0.000	13.111
126	8.140	0.258	0.000	5.215	0.000	0.000	0.243	0.000	13.855
128	7.663	0.289	0.000	5.010	0.000	0.000	0.240	0.000	13.202
130	7.829	0.294	0.000	4.624	0.000	0.000	0.256	0.000	13.003
132	7.499	0.277	0.000	4.508	0.000	0.000	0.254	0.000	12.538
134	7.752	0.336	0.000	4.743	0.000	0.000	0.263	0.000	13.094
136	7.541	0.340	0.000	4.549	0.000	0.000	0.252	0.000	12.682
138	7.817	0.367	0.000	4.430	0.000	0.000	0.279	0.000	12.893
138	7.687	0.369	0.000	4.394	0.000	0.000	0.275	0.000	12.725
142	7.092	0.309	0.000	4.406	0.000	0.000	0.289	0.000	12.096
144	6.412	0.279	0.000	3.831	0.000	0.000	0.249	0.000	10.771
152	6.430	0.247	0.000	3.731	0.000	0.000	0.282	0.000	10.690
154	6.711	0.254	0.000	4.186	0.000	0.000	0.259	0.000	11.410
156	6.065	0.236	0.000	4.175	0.000	0.000	0.269	0.000	10.745
158	6.650	0.250	0.000	4.835	0.000	0.000	0.281	0.000	12.016
160	6.795	0.240	0.000	4.655	0.000	0.000	0.256	0.000	11.946
162	7.138	0.282	0.000	4.909	0.000	0.000	0.277	0.000	12.607
164	7.376	0.254	0.000	4.635	0.000	0.000	0.299	0.000	12.563
166	7.215	0.249	0.000	4.633	0.000	0.000	0.335	0.000	12.432
168	6.760	0.259	0.000	4.486	0.000	0.000	0.316	0.000	11.820
170	6.246	0.225	0.000	3.954	0.000	0.000	0.298	0.000	10.723
172	7.867	0.301	0.000	4.563	0.000	0.000	0.326	0.000	13.058

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
174	8.204	0.305	0.000	4.608	0.000	0.000	0.338	0.000	13.455
176	8.842	0.283	0.000	4.406	0.000	0.000	0.368	0.000	13.900
178	7.388	0.219	0.000	3.856	0.058	0.000	0.301	0.000	11.823
180	7.649	0.262	0.071	4.363	0.069	0.000	0.308	0.000	12.722
182	8.221	0.236	0.091	4.979	0.082	0.000	0.300	0.000	13.909
184	9.284	0.361	0.000	5.167	0.086	0.000	0.248	0.000	15.146
186	8.457	0.305	0.000	4.900	0.062	0.000	0.235	0.000	13.959
188	7.968	0.247	0.000	4.745	0.069	0.000	0.217	0.000	13.245
192	7.427	0.244	0.000	4.922	0.062	0.000	0.207	0.000	12.862
194	7.245	0.196	0.000	4.883	0.063	0.000	0.180	0.000	12.567
196	8.055	0.259	0.000	4.936	0.056	0.000	0.166	0.000	13.473
198	8.610	0.255	0.000	4.622	0.056	0.000	0.151	0.000	13.695
200	7.625	0.232	0.000	3.574	0.000	0.000	0.125	0.000	11.556
202	8.866	0.285	0.000	4.536	0.000	0.000	0.188	0.000	13.875
204	8.170	0.285	0.000	4.870	0.000	0.000	0.260	0.000	13.585
206	8.379	0.341	0.000	5.060	0.000	0.000	0.298	0.000	14.078
208	7.036	0.361	0.000	4.591	0.000	0.000	0.308	0.000	12.295
210	7.394	0.367	0.000	4.904	0.061	0.000	0.340	0.000	13.066
212	6.551	0.360	0.000	5.107	0.065	0.000	0.383	0.000	12.466
214	5.398	0.407	0.000	5.526	0.067	0.000	0.406	0.000	11.804
218	6.235	0.502	0.000	5.604	0.067	0.000	0.470	0.000	12.878
230	9.892	0.696	0.000	6.660	0.075	0.068	0.635	0.000	18.026
236	8.109	0.626	0.000	6.621	0.085	0.062	0.605	0.000	16.109
240	7.076	0.557	0.000	7.087	0.106	0.073	0.632	0.000	15.531
244	5.126	0.457	0.070	5.956	0.118	0.067	0.592	0.081	12.468
246	5.133	0.533	0.000	6.018	0.123	0.060	0.546	0.077	12.489
248	4.802	0.500	0.000	6.828	0.155	0.059	0.594	0.000	12.938
250	3.749	0.361	0.110	5.251	0.136	0.058	0.497	0.000	10.162
252	4.906	0.413	0.132	6.200	0.144	0.065	0.590	0.000	12.450
254	6.272	0.394	0.146	6.296	0.162	0.053	0.603	0.000	13.926
256	6.939	0.398	0.000	6.227	0.159	0.054	0.643	0.000	14.420
258	7.096	0.409	0.150	6.507	0.150	0.056	0.708	0.000	15.075
260	7.077	0.424	0.156	6.514	0.157	0.058	0.804	0.000	15.190
262	6.155	0.335	0.000	6.510	0.152	0.058	0.813	0.000	14.022
264	5.996	0.271	0.151	6.252	0.130	0.052	0.771	0.000	13.623
266	6.310	0.296	0.142	6.502	0.120	0.051	0.820	0.000	14.241
268	6.526	0.310	0.125	5.833	0.104	0.000	0.806	0.105	13.809
270	6.826	0.309	0.000	5.631	0.103	0.000	0.820	0.083	13.771
274	7.046	0.507	0.099	5.572	0.000	0.000	0.815	0.000	14.039

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
276	6.015	0.555	0.000	4.854	0.069	0.055	0.791	0.000	12.339
278	5.971	0.642	0.061	4.823	0.061	0.066	0.836	0.000	12.460
280	5.420	0.680	0.000	4.487	0.050	0.068	0.783	0.000	11.488
282	5.344	0.754	0.000	4.398	0.000	0.071	0.729	0.000	11.297
284	5.932	0.927	0.000	4.363	0.081	0.077	0.606	0.000	11.986
286	7.242	1.016	0.067	4.748	0.103	0.085	0.616	0.000	13.878
288	7.943	1.073	0.078	4.806	0.090	0.078	0.621	0.000	14.689
290	7.122	0.824	0.072	3.870	0.097	0.074	0.485	0.000	12.544
292	9.255	0.877	0.000	4.697	0.109	0.000	0.552	0.000	15.490
294	8.291	0.754	0.000	4.779	0.113	0.000	0.555	0.000	14.491
296	9.154	0.712	0.103	4.633	0.140	0.066	0.494	0.124	15.427
298	8.999	0.618	0.000	5.041	0.132	0.000	0.594	0.000	15.383
300	8.932	0.550	0.107	5.127	0.125	0.064	0.533	0.000	15.438
302	8.031	0.512	0.000	5.009	0.109	0.000	0.514	0.131	14.305
304	8.425	0.481	0.000	5.320	0.108	0.000	0.478	0.271	15.082
306	8.622	0.429	0.000	5.705	0.107	0.000	0.489	0.000	15.352
308	9.486	0.418	0.000	5.969	0.114	0.000	0.554	0.000	16.541
310	9.487	0.410	0.000	5.460	0.103	0.000	0.584	0.000	16.043
312	9.478	0.384	0.000	5.222	0.000	0.000	0.620	0.000	15.704
314	10.093	0.391	0.000	5.245	0.000	0.000	0.624	0.000	16.352
316	9.640	0.408	0.000	5.540	0.000	0.000	0.641	0.000	16.229
318	9.623	0.383	0.000	5.444	0.000	0.000	0.554	0.000	16.003
320	9.400	0.357	0.000	5.578	0.000	0.000	0.533	0.000	15.869
322	10.093	0.366	0.000	5.970	0.000	0.000	0.628	0.000	17.055
324	10.354	0.317	0.000	5.823	0.000	0.000	0.698	0.000	17.193
326	9.985	0.284	0.000	5.455	0.000	0.000	0.641	0.125	16.490
328	9.497	0.262	0.000	5.135	0.000	0.000	0.611	0.000	15.506
330	9.226	0.231	0.000	5.203	0.000	0.000	0.607	0.000	15.266
332	8.534	0.202	0.000	4.766	0.000	0.000	0.583	0.000	14.084
334	9.132	0.241	0.000	5.018	0.000	0.000	0.651	0.000	15.042
336	8.335	0.205	0.000	4.506	0.000	0.000	0.567	0.000	13.613

Table M-2. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse countercurrent Fermentation CC (marine inocula, calcium carbonate buffer, LRT = 28.07 day, and VSLR = 4.50 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	3.004	0.000	0.000	0.281	0.000	0.000	0.000	0.000	3.285
2	4.397	0.081	0.000	1.196	0.000	0.000	0.000	0.000	5.674
4	5.090	0.086	0.000	2.106	0.000	0.000	0.054	0.000	7.337
6	5.668	0.099	0.000	2.555	0.000	0.000	0.086	0.000	8.409
8	5.926	0.151	0.000	2.873	0.000	0.000	0.168	0.000	9.118
10	5.566	0.132	0.000	2.733	0.000	0.000	0.180	0.000	8.611
16	7.613	0.264	0.000	3.555	0.000	0.000	0.306	0.000	11.738
23	8.426	0.252	0.000	3.742	0.000	0.000	0.309	0.000	12.729
26	8.364	0.210	0.000	3.766	0.000	0.000	0.328	0.000	12.668
27	7.405	0.171	0.000	3.571	0.000	0.000	0.323	0.000	11.47
30	11.388	0.305	0.000	4.920	0.000	0.000	0.598	0.000	17.211
31	10.236	0.197	0.000	4.755	0.000	0.000	0.407	0.000	15.594
35	9.879	0.180	0.000	4.257	0.000	0.000	0.469	0.000	14.786
41	12.495	0.343	0.000	4.910	0.000	0.000	0.586	0.000	18.333
42	12.285	0.302	0.000	4.827	0.000	0.000	0.579	0.000	17.992
58	20.094	0.513	0.000	5.326	0.000	0.000	0.486	0.000	26.418
60	19.232	0.459	0.068	5.223	0.000	0.000	0.453	0.000	25.435
62	18.292	0.425	0.075	5.438	0.000	0.000	0.484	0.000	24.714
64	17.159	0.370	0.085	5.789	0.053	0.000	0.463	0.000	23.92
66	16.228	0.352	0.000	5.783	0.000	0.000	0.383	0.000	22.746
68	17.043	0.411	0.091	6.518	0.056	0.000	0.311	0.000	24.43
70	14.852	0.350	0.000	6.516	0.000	0.000	0.289	0.000	22.007
76	15.278	0.480	0.065	7.100	0.000	0.056	0.503	0.000	23.483
78	15.280	0.403	0.066	7.584	0.000	0.000	0.484	0.000	23.817
80	13.754	0.355	0.064	7.452	0.000	0.000	0.511	0.000	22.137
84	14.003	0.404	0.059	8.053	0.000	0.000	0.646	0.000	23.165
86	12.806	0.319	0.057	7.479	0.000	0.000	0.571	0.000	21.232
88	12.713	0.292	0.062	7.474	0.000	0.000	0.563	0.000	21.103
90	12.447	0.343	0.062	7.567	0.000	0.000	0.625	0.000	21.044
92	12.051	0.332	0.064	7.292	0.051	0.000	0.607	0.000	20.398
94	11.550	0.327	0.062	6.765	0.000	0.000	0.631	0.000	19.335
96	12.448	0.340	0.061	6.386	0.000	0.000	0.629	0.000	19.864
98	12.765	0.313	0.000	6.737	0.000	0.000	0.631	0.000	20.445
100	13.295	0.338	0.059	6.854	0.000	0.000	0.686	0.000	21.231
102	13.306	0.325	0.000	6.429	0.000	0.000	0.637	0.000	20.697
104	12.996	0.287	0.000	6.677	0.000	0.000	0.616	0.000	20.575

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
106	12.318	0.276	0.000	6.974	0.000	0.000	0.716	0.000	20.284
108	12.007	0.253	0.000	6.498	0.000	0.000	0.678	0.000	19.437
110	11.287	0.218	0.000	6.492	0.000	0.000	0.652	0.000	18.65
112	12.221	0.231	0.056	7.246	0.000	0.000	0.727	0.000	20.481
114	11.443	0.209	0.000	7.241	0.000	0.000	0.719	0.000	19.612
116	7.528	0.172	0.000	3.518	0.000	0.000	0.286	0.000	11.505
118	12.104	0.229	0.067	8.103	0.055	0.000	0.935	0.000	21.493
120	12.554	0.239	0.070	8.024	0.055	0.000	0.921	0.000	21.862
124	10.747	0.204	0.000	7.032	0.055	0.000	0.850	0.000	18.887
126	10.461	0.193	0.000	7.300	0.053	0.000	0.921	0.000	18.927
128	9.869	0.182	0.067	6.967	0.000	0.000	0.888	0.000	17.973
130	15.284	0.458	0.146	4.985	0.129	0.000	0.096	0.000	21.098
132	10.468	0.187	0.000	6.832	0.000	0.000	0.788	0.000	18.276
134	10.289	0.200	0.000	7.303	0.000	0.000	0.828	0.000	18.619
136	10.450	0.207	0.059	7.269	0.000	0.000	0.835	0.000	18.82
138	10.379	0.208	0.061	6.979	0.000	0.000	0.850	0.000	18.477
138	9.934	0.209	0.000	6.811	0.000	0.000	0.829	0.000	17.782
142	9.412	0.189	0.000	6.356	0.000	0.000	0.776	0.000	16.733
148	10.067	0.178	0.000	6.720	0.000	0.000	0.724	0.000	17.689
150	10.005	0.217	0.000	7.043	0.000	0.000	0.760	0.000	18.025
152	8.908	0.220	0.000	6.647	0.000	0.000	0.659	0.000	16.434
154	8.955	0.215	0.000	7.101	0.000	0.000	0.563	0.000	16.834
156	9.300	0.227	0.000	7.896	0.051	0.000	0.519	0.000	17.993
158	9.232	0.251	0.000	7.700	0.000	0.000	0.502	0.000	17.686
160	9.470	0.235	0.000	7.911	0.000	0.000	0.523	0.000	18.139
162	9.669	0.232	0.000	7.856	0.000	0.000	0.533	0.000	18.289
164	9.430	0.197	0.075	7.562	0.000	0.000	0.534	0.000	17.797
166	9.546	0.197	0.000	7.260	0.000	0.000	0.556	0.000	17.559
168	10.252	0.236	0.079	7.484	0.055	0.000	0.562	0.000	18.669
172	10.929	0.252	0.082	8.005	0.067	0.000	0.612	0.000	19.948
172	11.505	0.258	0.081	8.024	0.057	0.000	0.641	0.000	20.566
176	11.472	0.250	0.081	7.683	0.060	0.000	0.640	0.000	20.186
178	10.762	0.200	0.081	7.133	0.061	0.000	0.519	0.000	18.756
180	10.494	0.190	0.077	6.742	0.057	0.000	0.540	0.000	18.099
182	10.076	0.179	0.077	6.453	0.055	0.000	0.513	0.000	17.354
184	10.539	0.178	0.000	6.422	0.056	0.000	0.568	0.000	17.762
186	10.667	0.194	0.070	6.423	0.000	0.000	0.583	0.000	17.936
188	10.808	0.209	0.065	6.707	0.050	0.000	0.679	0.000	18.518
191	11.635	0.228	0.062	6.758	0.000	0.000	0.747	0.000	19.43

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
193	10.876	0.215	0.061	6.247	0.054	0.000	0.679	0.000	18.133
195	10.775	0.221	0.061	6.072	0.000	0.000	0.780	0.000	17.909
197	11.359	0.232	0.060	5.702	0.000	0.000	0.769	0.000	18.122
199	10.366	0.199	0.057	5.458	0.000	0.000	0.695	0.000	16.775
201	10.251	0.214	0.056	5.376	0.000	0.000	0.663	0.000	16.56
203	11.190	0.261	0.064	5.622	0.053	0.000	0.660	0.000	17.849
205	8.591	0.197	0.000	4.546	0.000	0.000	0.488	0.000	13.822
207	10.398	0.299	0.000	5.482	0.052	0.000	0.630	0.000	16.861
209	10.678	0.292	0.000	5.518	0.054	0.000	0.656	0.000	17.199
211	10.758	0.292	0.000	6.433	0.059	0.000	0.664	0.000	18.205
213	11.336	0.306	0.000	6.954	0.063	0.000	0.784	0.000	19.443

Table M-3. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse countercurrent Fermentation CE (marine inocula, calcium carbonate buffer, LRT = 42.26 day, and VSLR = 6.24 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	10.142	0.276	0.000	4.947	0.061	0.000	1.087	0.000	16.512
4	9.793	0.288	0.060	4.983	0.060	0.054	1.246	0.000	16.484
39	10.779	0.348	0.000	6.290	0.000	0.062	1.428	0.000	18.908
41	11.224	0.362	0.000	6.422	0.000	0.067	1.463	0.000	19.538
49	12.433	0.385	0.000	6.785	0.052	0.070	1.541	0.063	21.329
53	12.892	0.387	0.000	6.832	0.000	0.000	1.591	0.000	21.702
61	13.329	0.379	0.000	6.890	0.000	0.000	1.539	0.000	22.137
71	18.023	0.539	0.000	9.043	0.000	0.000	2.112	0.000	29.717
73	17.806	0.508	0.000	7.955	0.000	0.000	1.874	0.000	28.143
75	19.870	0.542	0.000	8.414	0.000	0.000	2.073	0.000	30.899
77	19.233	0.525	0.000	7.876	0.000	0.000	1.944	0.000	29.578
79	19.456	0.510	0.000	7.692	0.000	0.000	1.879	0.000	29.537
81	19.721	0.504	0.000	7.908	0.000	0.000	1.834	0.000	29.967
85	18.399	0.461	0.000	7.358	0.000	0.000	1.602	0.000	27.819
89	17.457	0.407	0.000	7.741	0.000	0.000	1.570	0.000	27.175
91	17.727	0.407	0.000	7.420	0.000	0.083	1.477	0.000	27.114
93	18.010	0.401	0.000	7.310	0.000	0.000	1.471	0.000	27.191
95	18.452	0.386	0.000	7.311	0.000	0.000	1.471	0.000	27.62
97	17.643	0.365	0.000	6.649	0.000	0.000	1.328	0.101	26.086
99	18.040	0.367	0.000	6.989	0.000	0.000	1.368	0.157	26.921
101	19.451	0.360	0.000	6.757	0.000	0.000	1.336	0.000	27.904
103	18.917	0.347	0.000	6.686	0.000	0.000	1.379	0.000	27.329
105	18.449	0.336	0.000	6.283	0.000	0.000	1.267	0.000	26.334
107	17.777	0.319	0.000	5.944	0.000	0.000	1.283	0.000	25.322
109	18.592	0.325	0.000	5.760	0.000	0.000	1.193	0.000	25.87
111	19.268	0.335	0.000	5.898	0.000	0.000	1.170	0.000	26.671
113	18.824	0.326	0.000	6.256	0.000	0.000	1.137	0.000	26.542
115	18.470	0.308	0.000	6.203	0.000	0.000	1.061	0.000	26.042
117	17.857	0.311	0.000	7.474	0.000	0.000	1.130	0.000	26.773
119	17.680	0.303	0.000	7.027	0.000	0.000	1.085	0.000	26.096
121	18.205	0.316	0.000	7.449	0.000	0.000	1.199	0.000	27.169
123	16.873	0.300	0.000	7.333	0.000	0.000	1.158	0.000	25.664
125	18.451	0.319	0.000	7.304	0.000	0.000	1.135	0.000	27.209
127	18.621	0.323	0.000	7.644	0.000	0.000	1.225	0.000	27.812
129	18.511	0.324	0.000	7.875	0.000	0.000	1.217	0.000	27.926
131	18.925	0.346	0.000	7.226	0.000	0.000	1.058	0.000	27.554

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
133	18.328	0.350	0.000	6.945	0.000	0.000	1.034	0.000	26.658
135	18.607	0.344	0.000	7.524	0.000	0.000	1.183	0.000	27.659
137	17.510	0.338	0.000	6.858	0.000	0.000	0.981	0.000	25.687
139	18.931	0.383	0.000	7.109	0.000	0.000	1.019	0.000	27.442
141	18.362	0.371	0.000	7.716	0.000	0.000	1.131	0.000	27.58
143	19.155	0.389	0.000	7.597	0.000	0.000	1.070	0.000	28.211
145	18.970	0.380	0.000	7.722	0.000	0.000	1.127	0.336	28.537
147	19.153	0.374	0.000	8.025	0.000	0.000	1.190	0.000	28.742
149	18.775	0.402	0.000	7.717	0.000	0.000	1.068	0.000	27.963
153	19.491	0.380	0.000	7.981	0.000	0.000	1.298	0.000	29.15
157	19.824	0.381	0.000	7.406	0.000	0.000	1.285	0.000	28.896
157	19.845	0.381	0.000	7.406	0.000	0.000	1.292	0.000	28.924
159	20.026	0.400	0.000	8.223	0.000	0.000	0.000	0.000	28.648

Table M-4. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse countercurrent Fermentation CF (marine inocula, calcium carbonate buffer, LRT = 27.27 day, and VSLR = 4.85 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	9.658	0.336	0.000	5.885	0.059	0.062	1.161	0.000	17.16
7	10.445	0.343	0.000	6.232	0.000	0.066	1.381	0.000	18.467
9	10.800	0.358	0.000	6.351	0.062	0.071	1.414	0.000	19.056
17	11.717	0.365	0.000	6.946	0.000	0.072	1.539	0.000	20.64
21	12.341	0.369	0.000	6.977	0.000	0.000	1.579	0.000	21.266
39	18.494	0.457	0.000	10.747	0.000	0.106	2.192	0.000	31.997
41	21.889	0.594	0.000	10.433	0.000	0.117	2.137	0.000	35.17
43	20.817	0.591	0.000	10.173	0.000	0.000	1.890	0.000	33.47
45	23.508	0.677	0.000	11.933	0.000	0.000	1.983	0.000	38.1
53	24.478	0.608	0.000	11.240	0.000	0.000	1.804	0.000	38.13
59	23.082	0.540	0.000	10.360	0.000	0.103	1.877	0.000	35.962
61	21.801	0.513	0.000	9.852	0.000	0.000	1.791	0.104	34.061
63	19.697	0.464	0.000	8.989	0.000	0.000	1.681	0.000	30.831
65	20.327	0.463	0.000	9.009	0.000	0.000	1.678	0.000	31.479
67	20.196	0.436	0.000	8.636	0.000	0.000	1.663	0.000	30.932
69	19.437	0.400	0.000	8.054	0.000	0.000	1.576	0.000	29.467
73	17.542	0.340	0.000	7.554	0.000	0.000	1.538	0.000	26.974
75	17.280	0.327	0.000	7.355	0.000	0.000	1.532	0.000	26.494
77	16.931	0.323	0.000	7.469	0.000	0.000	1.465	0.000	26.188
79	16.041	0.297	0.000	6.929	0.000	0.000	1.358	0.117	24.742
81	14.974	0.277	0.000	7.431	0.000	0.000	1.450	0.000	24.133
83	15.000	0.265	0.000	6.894	0.000	0.000	1.350	0.000	23.51
85	14.852	0.263	0.000	7.610	0.000	0.000	1.545	0.000	24.27
87	14.276	0.259	0.000	7.308	0.000	0.000	1.468	0.000	23.312
89	13.846	0.249	0.000	7.071	0.000	0.000	1.464	0.000	22.63
91	14.152	0.266	0.000	6.902	0.000	0.000	1.517	0.000	22.838
93	14.685	0.291	0.000	6.796	0.000	0.000	1.381	0.000	23.153
95	14.127	0.267	0.000	6.303	0.000	0.000	1.300	0.000	21.998
97	14.285	0.287	0.000	6.540	0.000	0.000	1.333	0.000	22.445
99	13.672	0.275	0.000	6.223	0.000	0.000	1.231	0.000	21.401
101	14.485	0.289	0.000	6.418	0.000	0.000	1.290	0.000	22.483
103	14.408	0.294	0.000	6.194	0.000	0.000	1.259	0.000	22.155
105	14.776	0.304	0.000	6.077	0.000	0.000	1.277	0.000	22.434
107	14.681	0.294	0.000	5.432	0.000	0.000	1.079	0.000	21.485
109	14.415	0.303	0.000	6.044	0.000	0.000	1.163	0.000	21.925

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
111	13.519	0.297	0.000	5.368	0.000	0.000	0.986	0.000	20.17
113	13.911	0.325	0.000	5.826	0.000	0.000	1.037	0.000	21.099
115	13.807	0.335	0.000	5.767	0.000	0.000	1.014	0.000	20.923
117	13.908	0.334	0.000	6.200	0.000	0.000	0.998	0.000	21.441
119	13.970	0.344	0.000	5.930	0.000	0.000	1.006	0.000	21.25
121	14.001	0.359	0.000	6.198	0.000	0.000	1.042	0.000	21.6
123	14.012	0.344	0.000	6.067	0.000	0.000	1.094	0.000	21.517
125	13.516	0.334	0.000	6.067	0.000	0.000	0.990	0.000	20.907
127	13.286	0.336	0.000	6.044	0.000	0.000	0.000	0.000	19.665

APPENDIX N

CARBOXYLIC ACID PRODUCTION DATA FOR HOT-LIME-WATER-TREATED BAGASSE COUNTERCURRENT FERMENTATIONS BUFFERED BY AMMONIUM BICARBONATE

Table N-1. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse countercurrent Fermentation MA (marine inocula, ammonium bicarbonate buffer, LRT = 19.10 day, and VSLR = 2.07 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	3.207	0.000	0.000	0.223	0.000	0.000	0.000	0.000	3.43
2	4.065	0.000	0.000	0.895	0.000	0.000	0.000	0.000	4.961
4	5.708	0.000	0.000	1.371	0.000	0.000	0.000	0.000	7.08
6	6.982	0.176	0.000	1.786	0.000	0.000	0.000	0.000	8.945
12	10.595	0.342	0.000	2.112	0.000	0.000	0.000	0.000	13.049
14	11.899	0.313	0.139	2.165	0.093	0.000	0.000	0.000	14.609
16	12.952	0.352	0.167	2.184	0.110	0.000	0.000	0.000	15.765
18	13.797	0.350	0.187	2.236	0.119	0.000	0.000	0.000	16.688
20	14.618	0.340	0.217	2.371	0.133	0.000	0.000	0.000	17.679
22	15.239	0.311	0.244	2.416	0.150	0.000	0.000	0.000	18.36
24	15.347	0.292	0.250	2.413	0.153	0.000	0.000	0.000	18.455
26	15.976	0.299	0.270	2.439	0.164	0.000	0.000	0.000	19.148
29	18.250	0.349	0.239	2.890	0.146	0.000	0.000	0.000	21.874
31	16.527	0.361	0.000	2.702	0.112	0.000	0.000	0.000	19.703
33	18.478	0.409	0.200	2.592	0.112	0.000	0.000	0.000	21.792
35	19.423	0.519	0.190	2.433	0.105	0.000	0.000	0.000	22.67
40	19.668	0.562	0.180	4.408	0.114	0.000	0.000	0.000	24.932
44	20.559	0.599	0.154	3.915	0.113	0.000	0.000	0.000	25.34
46	18.631	0.530	0.142	2.729	0.113	0.000	0.000	0.000	22.146
48	20.873	0.605	0.180	2.807	0.150	0.000	0.000	0.000	24.616
50	18.592	0.532	0.166	2.875	0.128	0.000	0.000	0.000	22.293
52	19.464	0.564	0.171	2.685	0.115	0.000	0.000	0.000	22.998
54	19.748	0.608	0.161	2.603	0.113	0.000	0.000	0.000	23.234
58	17.906	0.356	0.118	1.922	0.090	0.000	0.000	0.000	20.391
60	18.198	0.396	0.000	2.036	0.083	0.000	0.000	0.000	20.713
62	17.057	0.383	0.097	1.875	0.067	0.000	0.000	0.000	19.48
62	18.570	0.384	0.098	1.916	0.066	0.000	0.000	0.000	21.033
64	18.977	0.388	0.081	1.738	0.050	0.000	0.000	0.000	21.235

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
66	15.675	0.294	0.000	1.351	0.000	0.000	0.000	0.000	17.319
68	14.245	0.255	0.000	1.152	0.000	0.000	0.000	0.000	15.652
70	15.646	0.749	0.383	1.569	0.000	0.000	0.000	0.000	18.347
72	15.796	0.280	0.000	1.477	0.000	0.000	0.000	0.000	17.552
74	14.930	0.615	0.118	1.388	0.000	0.000	0.000	0.000	17.052
76	14.607	0.762	0.162	1.343	0.000	0.000	0.000	0.000	16.874
78	14.740	0.224	0.000	1.410	0.000	0.000	0.000	0.000	16.374
80	15.488	0.727	0.290	1.605	0.000	0.000	0.000	0.000	18.111
82	16.710	0.362	0.000	1.338	0.000	0.000	0.000	0.000	18.41
86	13.815	0.248	0.000	1.332	0.000	0.000	0.000	0.000	15.395
88	12.525	0.273	0.000	1.264	0.000	0.000	0.000	0.000	14.063
90	12.896	0.148	0.000	1.151	0.000	0.000	0.000	0.000	14.194
92	13.112	0.284	0.000	1.221	0.000	0.000	0.000	0.000	14.617
94	12.828	0.883	0.099	1.233	0.000	0.000	0.000	0.000	15.043
96	12.380	0.113	0.000	1.144	0.000	0.000	0.000	0.000	13.637
98	11.898	1.013	0.082	1.090	0.000	0.000	0.000	0.000	14.083
100	11.794	0.084	0.000	1.153	0.000	0.000	0.000	0.000	13.031
104	9.153	0.269	0.000	1.051	0.000	0.000	0.000	0.000	10.473
106	9.210	0.316	0.000	1.369	0.000	0.000	0.000	0.000	10.895
116	9.145	0.385	0.000	0.993	0.069	0.000	0.000	0.000	10.593
120	9.897	0.303	0.076	0.810	0.059	0.000	0.000	0.000	11.145
122	10.375	0.234	0.000	0.849	0.000	0.000	0.000	0.000	11.458
124	11.715	0.250	0.000	0.962	0.000	0.000	0.000	0.000	12.926
126	14.626	0.377	0.097	0.876	0.055	0.000	0.000	0.000	16.031
128	13.104	0.284	0.000	0.664	0.089	0.000	0.000	0.000	14.141
130	13.011	0.325	0.000	0.582	0.139	0.000	0.000	0.000	14.058
132	13.020	0.291	0.145	0.485	0.154	0.000	0.000	0.000	14.095
134	14.200	0.355	0.000	0.912	0.163	0.000	0.000	0.000	15.631
136	13.965	0.245	0.000	0.960	0.147	0.000	0.000	0.000	15.317
138	13.915	0.223	0.000	0.973	0.092	0.000	0.000	0.000	15.204
140	12.926	0.218	0.068	1.017	0.060	0.000	0.000	0.000	14.288
142	13.946	0.256	0.089	0.967	0.085	0.000	0.000	0.000	15.344
146	12.530	0.239	0.000	1.161	0.000	0.000	0.000	0.000	13.93
148	13.254	0.254	0.066	1.240	0.060	0.000	0.000	0.000	14.874
148	12.369	0.245	0.000	1.186	0.000	0.000	0.000	0.000	13.8
150	12.600	0.291	0.060	1.153	0.080	0.000	0.000	0.000	14.183
152	12.711	0.301	0.074	1.273	0.096	0.000	0.000	0.000	14.454
154	12.116	0.269	0.060	1.289	0.081	0.000	0.000	0.000	13.814

Table N-2. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse countercurrent Fermentation MB (marine inocula, ammonium bicarbonate buffer, LRT = 19.26 day, and VSLR = 4.03 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	3.610	0.000	0.000	0.231	0.000	0.000	0.000	0.000	3.841
2	4.590	0.000	0.000	0.236	0.000	0.000	0.000	0.047	4.874
4	5.260	0.073	0.000	1.448	0.000	0.000	0.000	0.000	6.78
10	7.808	0.253	0.000	2.166	0.000	0.000	0.000	0.000	10.227
18	13.684	0.321	0.000	2.651	0.161	0.000	0.000	0.000	16.816
20	15.237	0.312	0.248	2.734	0.169	0.000	0.000	0.000	18.7
22	15.998	0.299	0.254	2.749	0.172	0.000	0.000	0.000	19.471
24	15.270	0.298	0.246	2.702	0.120	0.000	0.000	0.000	18.636
26	16.237	0.291	0.272	2.789	0.183	0.000	0.000	0.000	19.772
35	21.075	0.772	0.000	2.995	0.124	0.000	0.000	0.000	24.966
42	23.626	0.793	0.202	5.072	0.130	0.000	0.000	0.000	29.823
44	21.084	0.720	0.187	5.136	0.131	0.000	0.000	0.000	27.258
46	21.491	0.809	0.000	3.755	0.118	0.000	0.000	0.000	26.173
48	18.546	0.679	0.176	5.210	0.124	0.000	0.000	0.000	24.736
50	19.973	0.787	0.000	4.871	0.129	0.000	0.000	0.000	25.76
52	20.224	0.742	0.162	3.874	0.109	0.000	0.000	0.000	25.11
54	20.830	0.817	0.000	3.764	0.111	0.000	0.000	0.000	25.522
58	22.342	0.761	0.000	4.463	0.129	0.000	0.000	0.000	27.695
60	23.446	0.960	0.158	6.881	0.133	0.000	0.000	0.000	31.578
62	21.421	1.030	0.000	6.829	0.123	0.000	0.000	0.000	29.403
64	20.455	1.279	0.268	5.612	0.125	0.000	0.000	0.000	27.738
66	20.998	0.732	0.000	5.093	0.130	0.000	0.000	0.000	26.953
68	21.436	1.368	0.316	4.886	0.140	0.000	0.000	0.000	28.146
70	22.768	1.368	0.352	5.191	0.143	0.000	0.000	0.000	29.822
72	21.246	1.435	0.333	5.480	0.140	0.000	0.000	0.000	28.633
74	21.371	0.775	0.194	5.012	0.142	0.000	0.000	0.000	27.494
76	22.649	0.761	0.211	4.562	0.151	0.000	0.000	0.000	28.334
78	21.870	1.250	0.330	4.453	0.149	0.000	0.000	0.000	28.053
80	20.980	1.043	0.299	3.766	0.142	0.000	0.000	0.000	26.23
82	21.657	0.818	0.194	4.342	0.141	0.000	0.000	0.000	27.154
84	22.011	0.769	0.205	3.749	0.140	0.000	0.000	0.000	26.874
86	22.729	0.759	0.207	2.813	0.151	0.000	0.000	0.000	26.66
88	19.200	0.735	0.185	4.080	0.138	0.000	0.000	0.000	24.338
92	21.667	0.882	0.214	3.742	0.146	0.000	0.000	0.000	26.65
94	21.449	1.508	0.295	5.023	0.151	0.000	0.000	0.000	28.426
96	21.533	1.437	0.300	4.367	0.155	0.000	0.000	0.000	27.792

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
98	22.689	1.751	0.336	3.913	0.162	0.000	0.000	0.000	28.851
100	22.163	0.820	0.195	3.764	0.147	0.000	0.000	0.000	27.089
102	20.917	0.833	0.000	3.476	0.151	0.000	0.000	0.000	25.378
104	21.564	0.958	0.196	6.104	0.152	0.000	0.000	0.000	28.975
106	19.957	0.887	0.173	5.701	0.130	0.000	0.000	0.000	26.848
110	17.400	0.738	0.171	6.289	0.152	0.000	0.000	0.000	24.75
112	19.076	0.752	0.179	5.335	0.156	0.000	0.000	0.000	25.497
114	21.799	0.982	0.206	4.108	0.175	0.000	0.000	0.000	27.27
116	21.305	0.880	0.198	2.888	0.166	0.000	0.000	0.000	25.438
118	18.826	0.877	0.000	3.975	0.155	0.000	0.000	0.000	23.832
120	18.538	0.764	0.194	4.280	0.139	0.000	0.000	0.000	23.915
122	16.536	0.586	0.000	3.572	0.136	0.000	0.000	0.000	20.831
126	19.680	0.734	0.183	3.312	0.128	0.000	0.000	0.000	24.037
128	18.711	0.572	0.169	3.730	0.135	0.000	0.000	0.000	23.317
130	16.466	0.553	0.151	4.605	0.118	0.000	0.000	0.000	21.893
132	15.535	0.480	0.117	5.100	0.113	0.000	0.000	0.000	21.344
134	17.379	0.548	0.121	4.969	0.130	0.000	0.000	0.000	23.147
136	17.211	0.521	0.116	4.384	0.122	0.000	0.000	0.000	22.353
138	18.013	0.535	0.109	4.253	0.115	0.000	0.000	0.000	23.025
142	18.791	0.646	0.120	4.758	0.129	0.000	0.000	0.000	24.444
146	23.395	0.876	0.193	4.461	0.155	0.000	0.000	0.000	29.08
148	22.666	0.865	0.187	3.094	0.137	0.000	0.000	0.000	26.95
150	21.001	0.737	0.179	3.409	0.131	0.000	0.000	0.000	25.457
152	18.667	0.722	0.174	3.826	0.135	0.000	0.000	0.000	23.525
154	16.126	0.607	0.159	4.752	0.125	0.000	0.000	0.000	21.769
156	9.689	0.181	0.063	5.923	0.000	0.000	0.748	0.000	16.604
158	12.835	0.338	0.000	4.044	0.096	0.000	0.049	0.000	17.363
160	15.763	0.368	0.111	4.687	0.114	0.000	0.048	0.000	21.09
162	13.153	0.287	0.000	4.849	0.000	0.000	0.203	0.000	18.491
164	12.530	0.307	0.076	5.192	0.081	0.000	0.438	0.000	18.624
166	10.398	0.298	0.000	4.663	0.000	0.000	0.253	0.000	15.611
172	14.102	0.350	0.000	4.344	0.000	0.000	0.100	0.000	18.896
174	12.590	0.339	0.000	4.812	0.000	0.000	0.158	0.000	17.899
176	10.572	0.359	0.000	5.172	0.000	0.000	0.261	0.000	16.365
178	8.959	0.392	0.000	4.046	0.000	0.000	0.228	0.000	13.625
182	8.746	0.399	0.000	5.326	0.000	0.000	0.217	0.000	14.688
184	11.521	0.435	0.000	5.538	0.000	0.000	0.134	0.000	17.629
186	12.565	0.518	0.000	5.761	0.000	0.000	0.000	0.000	18.845
188	15.496	0.777	0.000	6.283	0.140	0.000	0.000	0.000	22.695

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
192	15.327	0.673	0.143	7.313	0.118	0.000	0.000	0.000	23.573
194	15.336	0.735	0.149	6.677	0.128	0.000	0.059	0.000	23.084
196	15.302	0.584	0.144	5.343	0.126	0.000	0.000	0.000	21.499
200	15.799	0.633	0.166	5.544	0.172	0.000	0.000	0.000	22.314
202	17.196	0.526	0.160	5.500	0.139	0.000	0.000	0.000	23.521
204	15.734	0.447	0.118	4.511	0.102	0.000	0.000	0.000	20.912
206	14.700	0.456	0.099	5.645	0.085	0.000	0.000	0.000	20.985
208	12.829	0.425	0.093	6.921	0.069	0.000	0.000	0.000	20.338
210	14.751	0.424	0.093	6.850	0.060	0.000	0.000	0.000	22.178
212	13.221	0.433	0.081	6.089	0.000	0.000	0.000	0.000	19.823
214	13.384	0.493	0.080	7.540	0.055	0.000	0.000	0.000	21.551
218	14.247	0.505	0.000	7.233	0.058	0.000	0.000	0.000	22.043
220	13.254	0.406	0.110	5.532	0.071	0.000	0.000	0.000	19.372
222	13.598	0.464	0.141	6.869	0.084	0.000	0.000	0.000	21.156
224	13.853	0.472	0.114	7.204	0.071	0.000	0.000	0.000	21.714
226	14.335	0.516	0.122	7.316	0.095	0.000	0.000	0.000	22.384
228	14.500	0.489	0.140	5.927	0.107	0.000	0.000	0.000	21.164
230	14.312	0.482	0.000	6.269	0.139	0.000	0.000	0.000	21.203
232	15.216	0.537	0.177	6.388	0.125	0.000	0.000	0.000	22.443
234	14.949	0.508	0.154	5.058	0.090	0.000	0.000	0.000	20.759
236	15.846	0.638	0.165	7.694	0.104	0.000	0.000	0.000	24.446
238	15.494	0.589	0.155	6.969	0.103	0.000	0.000	0.000	23.311
240	14.192	0.566	0.156	7.871	0.111	0.000	0.000	0.000	22.896
244	13.941	0.645	0.138	7.654	0.093	0.000	0.000	0.000	22.471
256	22.719	1.065	0.149	7.496	0.112	0.000	0.000	0.000	31.541
262	25.091	1.029	0.279	7.583	0.286	0.000	0.000	0.000	34.268
266	22.242	0.841	0.319	8.140	0.356	0.000	0.047	0.000	31.945
270	19.988	0.783	0.325	9.124	0.377	0.000	0.000	0.000	30.596
270	22.602	0.855	0.342	9.712	0.425	0.000	0.000	0.000	33.936
272	16.518	0.453	0.292	6.499	0.334	0.000	0.000	0.000	24.096
274	17.030	0.453	0.289	6.286	0.365	0.000	0.000	0.047	24.471
276	14.045	0.399	0.253	7.136	0.270	0.000	0.000	0.068	22.171
278	13.240	0.399	0.220	6.607	0.217	0.000	0.000	0.000	20.683
280	12.104	0.423	0.000	4.578	0.146	0.000	0.000	0.000	17.251
284	12.872	0.596	0.000	5.829	0.123	0.000	0.000	0.066	19.486
288	17.033	0.717	0.190	6.872	0.151	0.000	0.000	0.000	24.962
290	16.095	0.645	0.174	6.755	0.139	0.000	0.000	0.000	23.807
292	15.536	0.585	0.148	7.515	0.120	0.000	0.000	0.000	23.904
294	15.748	0.572	0.141	8.291	0.113	0.000	0.000	0.000	24.865

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
296	15.953	0.541	0.123	7.325	0.101	0.000	0.000	0.000	24.043
300	17.270	0.729	0.000	6.287	0.149	0.000	0.000	0.000	24.435
302	16.353	0.743	0.159	5.319	0.166	0.000	0.000	0.000	22.74
304	17.372	0.817	0.167	4.839	0.161	0.000	0.000	0.000	23.357
306	18.396	0.821	0.176	4.325	0.174	0.000	0.000	0.000	23.891
308	19.038	0.878	0.206	5.085	0.190	0.000	0.000	0.046	25.443
310	15.789	0.702	0.166	6.274	0.144	0.000	0.000	0.058	23.133
312	16.678	0.741	0.154	7.330	0.132	0.000	0.000	0.054	25.089
314	15.795	0.659	0.136	6.696	0.105	0.000	0.000	0.000	23.391
318	13.141	0.546	0.107	5.621	0.088	0.000	0.000	0.059	19.561
322	15.971	0.668	0.125	8.253	0.117	0.000	0.000	0.000	25.134
324	16.893	0.792	0.152	7.385	0.113	0.000	0.000	0.000	25.335
326	16.943	0.756	0.150	6.963	0.107	0.000	0.000	0.000	24.919
328	15.287	0.719	0.186	6.690	0.145	0.000	0.000	0.127	23.154
330	18.576	1.063	0.000	6.882	0.189	0.000	0.000	0.202	26.912
332	17.333	0.949	0.231	5.883	0.164	0.000	0.000	0.000	24.56
334	21.744	1.181	0.260	6.394	0.168	0.000	0.000	0.000	29.748
336	22.629	1.195	0.251	4.408	0.137	0.000	0.000	0.000	28.619
338	21.238	1.295	0.223	6.817	0.124	0.000	0.000	0.000	29.696
340	20.005	1.101	0.204	5.923	0.000	0.000	0.000	0.000	27.234
342	17.487	0.984	0.177	5.354	0.132	0.000	0.000	0.000	24.133
344	19.691	0.893	0.000	3.850	0.146	0.000	0.000	0.000	24.58
346	19.756	0.862	0.000	4.260	0.000	0.000	0.000	0.000	24.878
348	19.525	0.900	0.000	5.329	0.146	0.000	0.000	0.000	25.9
350	17.838	0.645	0.000	6.252	0.121	0.000	0.000	0.000	24.857
352	16.847	0.526	0.125	6.509	0.103	0.000	0.000	0.000	24.11
354	15.333	0.416	0.000	5.231	0.000	0.000	0.000	0.000	20.98
356	17.898	0.584	0.129	5.028	0.112	0.000	0.000	0.000	23.75
358	17.681	0.607	0.000	5.685	0.000	0.000	0.000	0.000	23.973
360	18.368	0.753	0.125	7.022	0.127	0.000	0.000	0.000	26.395
362	17.842	0.671	0.121	6.096	0.116	0.000	0.000	0.000	24.846
364	16.840	0.522	0.000	8.485	0.000	0.000	1.913	0.000	27.76
366	16.994	0.412	0.000	9.488	0.000	0.109	1.981	0.000	28.985
368	19.320	0.715	0.183	5.842	0.155	0.000	0.000	0.000	26.215
370	19.416	0.774	0.000	6.491	0.162	0.000	0.000	0.000	26.843
372	18.727	0.723	0.000	6.854	0.154	0.000	0.000	0.000	26.458
374	20.699	0.757	0.000	5.093	0.130	0.000	0.000	0.000	26.678

Table N-3. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse countercurrent Fermentation MC (marine inocula, ammonium bicarbonate buffer, LRT = 14.29 day, and VSLR = 3.32 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	2.041	0.000	0.000	0.164	0.000	0.000	0.000	0.000	2.205
2	6.295	0.108	0.000	1.922	0.000	0.000	0.000	0.000	8.325
4	7.855	0.138	0.000	2.747	0.000	0.000	0.107	0.000	10.847
6	8.436	0.250	0.000	2.846	0.000	0.000	0.109	0.000	11.642
8	9.531	0.340	0.142	3.000	0.000	0.000	0.101	0.000	13.115
16	14.284	0.742	0.430	4.775	0.233	0.000	0.000	0.000	20.464
26	17.930	0.820	0.525	5.149	0.300	0.000	0.088	0.000	24.813
28	18.352	0.822	0.545	5.229	0.297	0.000	0.000	0.000	25.246
30	18.410	0.837	0.514	5.051	0.221	0.000	0.000	0.000	25.033
32	20.329	0.891	0.541	5.344	0.245	0.000	0.088	0.000	27.437
34	20.335	0.889	0.539	5.321	0.247	0.000	0.085	0.000	27.418
37	22.491	0.589	0.222	2.961	0.134	0.000	0.000	0.000	26.397
45	24.044	0.897	0.564	5.308	0.254	0.000	0.096	0.000	31.164
47	24.185	0.871	0.573	5.344	0.258	0.000	0.097	0.000	31.328
52	25.682	0.840	0.558	5.412	0.241	0.000	0.093	0.000	32.826
53	26.284	0.812	0.561	5.508	0.238	0.000	0.094	0.000	33.497
55	30.879	0.863	0.303	3.307	0.143	0.000	0.059	0.000	35.555
55	37.236	1.042	0.355	4.009	0.180	0.000	0.055	0.000	42.878
57	31.583	0.897	0.396	4.233	0.193	0.000	0.064	0.000	37.367
59	33.065	0.788	0.385	4.114	0.184	0.000	0.000	0.000	38.536
61	27.637	1.589	0.527	3.200	0.141	0.000	0.000	0.000	33.094
63	21.821	0.753	0.246	5.806	0.135	0.000	0.000	0.000	28.761
65	17.658	0.684	0.186	5.692	0.114	0.000	0.000	0.000	24.334
67	14.777	0.420	0.000	5.637	0.099	0.000	0.000	0.000	20.932
69	13.850	0.413	0.000	4.342	0.099	0.000	0.000	0.000	18.704
71	13.145	0.328	0.000	3.557	0.074	0.000	0.000	0.000	17.103
75	14.425	0.402	0.000	2.553	0.000	0.000	0.000	0.000	17.381
77	13.963	0.390	0.000	2.280	0.000	0.000	0.000	0.000	16.633
79	13.923	0.608	0.076	1.542	0.066	0.000	0.000	0.000	16.214
81	13.756	0.560	0.000	1.505	0.066	0.000	0.000	0.000	15.888
87	12.004	0.418	0.000	2.989	0.000	0.000	0.000	0.000	15.41
89	11.630	0.511	0.000	2.060	0.000	0.000	0.000	0.000	14.201
91	14.015	0.597	0.000	1.921	0.116	0.000	0.000	0.000	16.649
93	12.803	0.532	0.166	2.369	0.168	0.000	0.000	0.000	16.038
95	12.580	0.502	0.199	2.581	0.186	0.000	0.000	0.000	16.047

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
99	15.711	0.547	0.213	3.137	0.203	0.000	0.000	0.000	19.811
101	12.545	0.424	0.000	3.604	0.114	0.000	0.075	0.000	16.763
103	12.786	0.412	0.125	4.415	0.081	0.000	0.055	0.000	17.873
105	10.805	0.339	0.130	3.345	0.109	0.000	0.000	0.000	14.728
107	9.640	0.458	0.000	7.243	0.071	0.000	0.094	0.000	17.506
109	8.136	0.424	0.000	6.348	0.063	0.000	0.184	0.000	15.155
111	8.001	0.377	0.063	5.164	0.074	0.000	0.080	0.000	13.759
113	8.818	0.408	0.077	4.956	0.072	0.000	0.064	0.000	14.396
115	8.681	0.389	0.000	5.660	0.000	0.000	0.066	0.000	14.797
117	8.291	0.396	0.000	5.582	0.000	0.000	0.167	0.000	14.436
119	7.692	0.321	0.000	4.844	0.000	0.000	0.259	0.000	13.116
123	9.815	0.434	0.000	3.571	0.082	0.000	0.082	0.000	13.983
125	10.231	0.517	0.142	5.422	0.000	0.000	0.000	0.000	16.312
127	11.367	0.475	0.000	4.792	0.000	0.000	0.056	0.000	16.689
133	7.999	0.292	0.000	3.890	0.000	0.000	0.158	0.000	12.338
141	10.211	0.375	0.000	3.404	0.000	0.000	0.000	0.000	13.99
143	9.667	0.393	0.000	3.691	0.000	0.000	0.000	0.000	13.751
147	8.698	0.446	0.000	4.965	0.000	0.000	0.168	0.000	14.276
153	8.823	0.335	0.000	3.459	0.000	0.000	0.000	0.000	12.617
155	9.707	0.357	0.000	3.090	0.000	0.000	0.000	0.000	13.154
157	10.218	0.427	0.000	3.466	0.000	0.000	0.000	0.000	14.111
159	10.389	0.375	0.000	3.151	0.000	0.000	0.000	0.000	13.915
161	11.956	0.459	0.000	3.090	0.000	0.000	0.000	0.000	15.505
163	13.294	0.522	0.000	3.028	0.000	0.000	0.000	0.000	16.844
165	11.709	0.423	0.000	2.926	0.000	0.000	0.000	0.000	15.058
167	12.663	0.417	0.085	2.750	0.058	0.000	0.000	0.000	15.973
169	11.730	0.344	0.000	3.583	0.000	0.000	0.000	0.000	15.657
171	12.375	0.413	0.068	3.259	0.054	0.000	0.000	0.000	16.168
173	13.055	0.427	0.116	2.977	0.072	0.000	0.000	0.000	16.648
175	13.762	0.440	0.137	2.882	0.102	0.000	0.000	0.000	17.323
179	14.642	0.442	0.161	2.593	0.125	0.000	0.000	0.000	17.963
181	13.630	0.411	0.157	2.766	0.105	0.000	0.000	0.000	17.068
185	15.373	0.529	0.186	3.199	0.136	0.000	0.000	0.000	19.422
187	13.209	0.375	0.177	3.203	0.133	0.000	0.000	0.000	17.096
189	13.005	0.385	0.151	2.475	0.101	0.000	0.000	0.000	16.117
191	13.301	0.465	0.185	1.486	0.141	0.000	0.000	0.000	15.578
193	11.423	0.457	0.225	0.965	0.000	0.000	0.000	0.000	13.071
195	11.977	0.503	0.232	0.811	0.140	0.000	0.000	0.064	13.728
197	14.430	0.536	0.168	1.115	0.116	0.000	0.000	0.000	16.365

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
199	14.734	0.547	0.139	1.232	0.103	0.000	0.000	0.000	16.754
203	16.444	0.668	0.000	1.521	0.121	0.000	0.000	0.000	18.754
205	19.412	0.832	0.236	1.673	0.245	0.000	0.000	0.000	22.398
207	16.799	0.759	0.206	1.645	0.216	0.000	0.000	0.000	19.625
211	16.570	0.805	0.208	2.080	0.195	0.000	0.000	0.000	19.858
229	9.974	1.143	0.270	2.552	0.302	0.000	0.000	0.000	14.241
233	8.631	0.607	0.241	1.610	0.278	0.000	0.000	0.000	11.366
237	9.798	0.589	0.229	2.911	0.283	0.000	0.000	0.000	13.811
239	8.058	0.242	0.201	2.148	0.275	0.000	0.000	0.000	10.924
241	7.372	0.241	0.164	2.515	0.225	0.000	0.000	0.000	10.518
243	7.776	0.307	0.145	3.015	0.185	0.000	0.000	0.044	11.472
245	8.613	0.342	0.107	2.505	0.113	0.000	0.000	0.000	11.680
247	10.349	0.451	0.132	2.578	0.145	0.000	0.000	0.000	13.654
249	9.191	0.431	0.112	2.428	0.105	0.000	0.000	0.062	12.328
251	10.917	0.414	0.103	3.296	0.087	0.000	0.000	0.000	14.816
253	11.492	0.417	0.090	2.871	0.076	0.000	0.000	0.000	14.946
255	11.721	0.476	0.107	3.207	0.088	0.000	0.000	0.000	15.598
257	11.907	0.490	0.099	3.602	0.090	0.000	0.000	0.000	16.188
259	13.661	0.585	0.135	3.440	0.110	0.000	0.000	0.000	17.931
261	12.958	0.544	0.155	2.825	0.160	0.000	0.000	0.000	16.643
263	11.074	0.443	0.111	2.833	0.105	0.000	0.000	0.000	14.566
267	14.683	0.644	0.000	2.278	0.097	0.000	0.000	0.000	17.702
269	12.905	0.569	0.093	2.125	0.090	0.000	0.000	0.000	15.782
271	13.155	0.610	0.131	1.737	0.124	0.000	0.000	0.000	15.757
273	12.852	0.606	0.106	1.846	0.099	0.000	0.000	0.000	15.508
275	12.978	0.612	0.152	3.273	0.114	0.000	0.000	0.000	17.129
277	10.280	0.448	0.117	2.779	0.096	0.000	0.000	0.000	13.720
279	11.476	0.461	0.120	3.705	0.105	0.000	0.000	0.000	15.867
281	10.817	0.392	0.108	3.103	0.091	0.000	0.000	0.000	14.511
283	12.238	0.454	0.119	3.148	0.105	0.000	0.000	0.000	16.064
285	12.095	0.408	0.000	3.145	0.088	0.000	0.000	0.000	15.737
289	12.510	0.355	0.094	2.764	0.099	0.000	0.000	0.000	15.821
291	13.146	0.481	0.133	2.457	0.101	0.000	0.000	0.000	16.319
293	16.256	0.667	0.227	1.843	0.202	0.000	0.000	0.000	19.196
295	14.107	0.619	0.000	1.220	0.245	0.000	0.000	0.000	16.190
297	15.466	0.811	0.000	1.946	0.261	0.000	0.000	0.000	18.484
299	14.456	0.667	0.000	2.004	0.241	0.000	0.000	0.000	17.368
301	16.382	0.686	0.272	2.858	0.223	0.000	0.000	0.000	20.421
303	13.563	0.488	0.222	2.456	0.185	0.000	0.000	0.000	16.913

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
305	14.509	0.513	0.215	2.718	0.17	0.000	0.000	0.000	18.125
307	14.407	0.475	0.187	2.534	0.133	0.000	0.000	0.000	17.736
309	14.544	0.554	0.175	3.198	0.140	0.000	0.000	0.000	18.611
311	9.967	0.321	0.000	2.408	0.000	0.000	0.000	0.000	12.696
313	12.271	0.379	0.130	3.451	0.000	0.000	0.000	0.000	16.232
315	13.258	0.410	0.132	3.717	0.112	0.000	0.000	0.000	17.629
317	11.084	0.322	0.000	3.679	0.000	0.000	0.000	0.000	15.084
319	14.594	0.442	0.116	3.788	0.000	0.000	0.000	0.000	18.941
321	14.744	0.428	0.161	3.513	0.117	0.000	0.000	0.000	18.962
323	14.717	0.474	0.178	3.974	0.125	0.000	0.000	0.000	19.469
325	12.980	0.436	0.181	3.643	0.143	0.000	0.000	0.000	17.383
327	11.060	0.345	0.136	3.056	0.120	0.000	0.000	0.000	14.717
329	12.640	0.394	0.171	3.264	0.131	0.000	0.000	0.000	16.601
335	12.154	0.356	0.000	3.088	0.000	0.000	0.000	0.000	15.598
337	13.853	0.416	0.000	3.065	0.000	0.000	0.000	0.000	17.335
339	14.741	0.450	0.118	3.244	0.000	0.000	0.000	0.000	18.553
341	13.227	0.426	0.116	2.714	0.000	0.000	0.000	0.000	16.483

Table N-4. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse countercurrent Fermentation MD (marine inocula, ammonium bicarbonate buffer, LRT = 26.26 day, and VSLR = 4.31 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	4.637	0.073	0.000	0.283	0.000	0.000	0.000	0.000	4.993
2	5.965	0.102	0.000	1.915	0.000	0.000	0.000	0.000	7.982
4	7.489	0.165	0.000	2.726	0.000	0.000	0.075	0.000	10.456
6	8.391	0.205	0.000	2.710	0.000	0.000	0.075	0.000	11.382
8	9.618	0.277	0.000	2.793	0.000	0.000	0.073	0.000	12.761
10	10.279	0.324	0.000	2.784	0.000	0.000	0.000	0.000	13.387
16	14.723	0.442	0.110	2.965	0.061	0.000	0.077	0.000	18.378
25	19.722	0.533	0.138	3.125	0.085	0.000	0.080	0.000	23.682
26	20.040	0.476	0.130	3.092	0.079	0.000	0.079	0.000	23.896
28	20.371	0.432	0.129	3.146	0.079	0.000	0.077	0.000	24.234
30	24.590	0.460	0.139	3.516	0.090	0.000	0.077	0.000	28.872
32	24.753	0.465	0.140	3.550	0.091	0.000	0.082	0.000	29.079
34	24.409	0.457	0.138	3.498	0.089	0.000	0.079	0.000	28.671
45	26.938	0.508	0.179	3.683	0.112	0.000	0.076	0.000	31.497
47	27.983	0.499	0.185	3.722	0.116	0.000	0.075	0.000	32.581
52	28.808	1.000	0.287	3.556	0.095	0.000	0.076	0.000	33.822
53	28.565	0.572	0.183	3.451	0.091	0.000	0.074	0.000	32.937
55	30.413	1.544	0.387	2.655	0.097	0.000	0.128	0.000	35.225
57	31.421	1.813	0.765	3.422	0.113	0.000	0.129	0.000	37.663
59	32.510	1.802	0.746	3.351	0.105	0.000	0.142	0.000	38.655
61	32.371	0.873	0.154	3.125	0.096	0.000	0.130	0.000	36.75
63	30.907	1.580	0.403	2.693	0.085	0.000	0.114	0.000	35.782
65	25.328	1.408	0.706	7.079	0.085	0.000	0.099	0.000	34.706
67	21.935	0.531	0.109	7.434	0.093	0.000	0.079	0.000	30.181
69	20.316	1.418	0.254	7.112	0.087	0.000	0.064	0.000	29.252
73	20.369	1.035	0.110	6.991	0.109	0.000	0.000	0.000	28.613
75	20.279	1.096	0.116	6.740	0.107	0.000	0.000	0.000	28.338
77	20.088	1.073	0.126	6.717	0.107	0.000	0.000	0.000	28.111
79	20.309	1.100	0.134	6.793	0.112	0.000	0.000	0.000	28.448
81	22.179	1.213	0.155	6.604	0.130	0.000	0.000	0.000	30.28
85	23.196	1.194	0.167	6.858	0.142	0.000	0.000	0.000	31.556
87	21.359	1.094	0.176	7.745	0.148	0.000	0.000	0.000	30.522
89	21.939	1.246	0.187	6.332	0.148	0.000	0.000	0.000	29.851
91	23.380	1.248	0.190	5.613	0.164	0.000	0.000	0.000	30.595
93	23.695	1.179	0.204	6.063	0.182	0.000	0.000	0.000	31.322
95	21.673	1.073	0.219	5.863	0.193	0.000	0.000	0.000	29.022

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
97	21.442	1.142	0.242	7.356	0.214	0.000	0.000	0.000	30.396
99	21.608	1.156	0.279	7.889	0.237	0.000	0.000	0.000	31.17
101	21.948	1.084	0.295	7.868	0.250	0.000	0.000	0.000	31.445
103	22.760	1.224	0.329	7.195	0.265	0.000	0.000	0.000	31.773
105	22.474	1.192	0.327	6.478	0.253	0.000	0.000	0.000	30.724
107	23.269	1.158	0.339	6.133	0.256	0.000	0.000	0.000	31.154
109	23.821	1.172	0.358	6.946	0.265	0.000	0.000	0.000	32.562
111	23.788	1.185	0.383	7.250	0.280	0.000	0.000	0.000	32.886
113	23.171	1.077	0.380	8.016	0.275	0.000	0.000	0.000	32.919
115	21.669	1.065	0.369	8.023	0.271	0.000	0.000	0.000	31.397
117	23.260	1.002	0.382	8.777	0.289	0.000	0.000	0.000	33.711
119	22.019	0.882	0.376	9.673	0.289	0.000	0.000	0.000	33.239
121	19.968	0.964	0.353	8.795	0.262	0.000	0.000	0.000	30.342
123	20.566	0.935	0.340	7.832	0.261	0.000	0.000	0.000	29.933
125	22.604	0.995	0.347	7.301	0.270	0.000	0.000	0.000	31.517
127	22.115	1.016	0.331	7.650	0.262	0.000	0.000	0.000	31.375
129	21.823	1.003	0.313	8.255	0.249	0.000	0.000	0.000	31.643
131	21.477	0.895	0.286	7.980	0.223	0.000	0.000	0.000	30.862

Table N-5. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse countercurrent Fermentation ME (marine inocula, ammonium bicarbonate buffer, LRT = 31.78 day, and VSLR = 5.50 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	5.518	0.089	0.000	0.324	0.000	0.000	0.000	0.000	5.931
2	6.713	0.154	0.000	2.463	0.000	0.000	0.000	0.000	9.33
4	7.012	0.191	0.000	2.881	0.000	0.000	0.000	0.000	10.085
6	7.948	0.212	0.000	2.967	0.000	0.000	0.000	0.000	11.127
8	9.580	0.285	0.090	3.135	0.064	0.000	0.000	0.000	13.154
10	10.504	0.332	0.104	3.154	0.075	0.000	0.000	0.000	14.169
16	13.445	0.401	0.135	3.226	0.073	0.000	0.000	0.000	17.281
18	13.946	0.408	0.128	3.190	0.063	0.000	0.000	0.000	17.736
25	20.008	0.542	0.147	3.314	0.086	0.000	0.000	0.000	24.097
26	21.621	0.529	0.151	3.410	0.097	0.000	0.000	0.000	25.808
28	18.891	0.710	0.402	4.629	0.206	0.000	0.151	0.000	24.989
30	23.114	0.460	0.149	3.292	0.096	0.000	0.000	0.000	27.111
32	21.686	0.434	0.000	3.110	0.000	0.000	0.000	0.000	25.229
39	24.660	0.481	0.152	3.407	0.104	0.000	0.000	0.000	28.803
45	28.949	0.547	0.191	3.585	0.120	0.000	0.000	0.000	33.392
47	29.071	0.521	0.194	3.596	0.121	0.000	0.000	0.000	33.503
52	31.016	0.549	0.193	3.672	0.117	0.000	0.000	0.000	35.547
53	29.791	1.085	0.277	3.460	0.101	0.000	0.000	0.000	34.713
55	29.835	0.849	0.000	3.401	0.099	0.000	0.133	0.000	34.317
57	29.448	1.787	0.523	3.967	0.113	0.000	0.102	0.000	35.94
59	28.844	1.868	0.514	3.975	0.109	0.000	0.118	0.000	35.428
61	29.481	1.727	0.684	3.677	0.102	0.000	0.111	0.000	35.783
63	26.623	1.569	0.391	5.552	0.098	0.000	0.104	0.000	34.337
65	23.844	1.441	0.640	8.231	0.096	0.000	0.092	0.000	34.343
67	22.932	0.691	0.163	8.554	0.111	0.000	0.075	0.000	32.525
69	22.466	0.714	0.143	8.557	0.115	0.000	0.000	0.000	31.994
71	20.700	0.725	0.132	9.354	0.115	0.000	0.057	0.000	31.083
73	18.480	0.711	0.122	9.659	0.121	0.000	0.052	0.000	29.145
75	19.883	0.820	0.122	10.475	0.126	0.000	0.000	0.000	31.427
77	21.177	0.831	0.125	10.235	0.108	0.000	0.000	0.000	32.475
79	19.131	0.776	0.000	8.957	0.000	0.000	0.000	0.000	28.863
81	20.410	1.288	0.124	9.387	0.097	0.000	0.000	0.000	31.306
85	23.646	1.495	0.166	10.472	0.141	0.000	0.000	0.000	35.92
87	25.516	1.341	0.192	10.201	0.192	0.000	0.000	0.000	37.442
89	24.147	1.332	0.214	13.596	0.253	0.000	0.000	0.000	39.542

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
91	23.901	1.223	0.222	11.958	0.247	0.000	0.000	0.000	37.551
93	22.767	1.077	0.230	10.795	0.180	0.000	0.000	0.000	35.049
95	23.312	1.137	0.253	12.394	0.222	0.000	0.000	0.000	37.318
97	22.105	1.298	0.238	11.209	0.242	0.000	0.000	0.000	35.093
99	21.623	1.070	0.247	9.830	0.235	0.000	0.000	0.000	33.005
101	23.597	1.058	0.267	11.028	0.262	0.000	0.000	0.000	36.212
103	21.892	0.898	0.258	10.644	0.226	0.000	0.000	0.000	33.918
105	21.703	0.880	0.256	11.269	0.225	0.000	0.000	0.000	34.333
107	21.683	0.830	0.246	12.088	0.245	0.000	0.000	0.000	35.092
109	20.647	0.746	0.237	12.623	0.255	0.000	0.000	0.000	34.508
111	19.701	0.888	0.236	12.103	0.161	0.000	0.000	0.000	33.089
113	19.967	1.104	0.000	11.627	0.162	0.000	0.000	0.000	32.861
115	20.645	1.120	0.228	11.809	0.168	0.000	0.000	0.000	33.970
117	21.731	1.078	0.213	11.581	0.167	0.000	0.000	0.000	34.770
119	22.444	0.980	0.198	13.095	0.171	0.000	0.000	0.000	36.887
123	18.714	0.743	0.000	12.866	0.131	0.000	0.000	0.000	32.454
125	18.322	0.662	0.167	13.291	0.192	0.000	0.000	0.000	32.633
127	19.264	0.613	0.159	13.022	0.219	0.000	0.047	0.000	33.323
129	19.661	0.665	0.164	14.061	0.224	0.000	0.000	0.000	34.775
131	17.621	0.666	0.000	13.435	0.131	0.000	0.000	0.000	31.853
133	17.639	0.653	0.000	13.279	0.132	0.000	0.000	0.000	31.703
135	16.589	0.612	0.000	13.494	0.130	0.000	0.000	0.000	30.825
143	17.662	1.139	0.185	14.087	0.163	0.000	0.000	0.000	33.236
145	17.321	0.664	0.000	7.225	0.000	0.000	0.253	0.000	25.463
147	18.932	1.109	0.194	13.818	0.174	0.000	0.000	0.000	34.228
151	17.107	0.840	0.000	14.127	0.186	0.000	0.000	0.000	32.259
153	16.151	0.726	0.206	14.503	0.172	0.000	0.000	0.000	31.758
155	17.353	0.761	0.231	15.281	0.188	0.000	0.000	0.000	33.813
157	18.469	0.761	0.220	13.710	0.174	0.000	0.000	0.000	33.333
161	18.541	0.719	0.211	13.365	0.166	0.000	0.000	0.000	33.002
163	19.198	0.724	0.209	13.029	0.165	0.000	0.000	0.000	33.325
165	20.795	0.746	0.218	12.385	0.179	0.000	0.000	0.000	34.323
167	22.798	0.819	0.245	14.044	0.221	0.000	0.059	0.000	38.186
167	22.184	0.777	0.228	13.094	0.183	0.000	0.051	0.154	36.672
169	23.511	0.815	0.233	11.382	0.194	0.000	0.048	0.191	36.375
171	24.812	0.866	0.249	12.013	0.245	0.000	0.000	0.000	38.185
173	24.062	0.830	0.247	11.310	0.210	0.060	0.000	0.000	36.719
175	23.250	0.831	0.253	11.821	0.205	0.000	0.000	0.000	36.360
179	22.569	0.814	0.254	12.309	0.201	0.000	0.000	0.000	36.147

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
181	23.292	0.789	0.249	11.369	0.213	0.000	0.000	0.000	35.911
185	23.928	0.818	0.260	12.473	0.207	0.000	0.000	0.000	37.686
187	23.936	0.830	0.268	11.641	0.205	0.000	0.000	0.000	36.880
189	25.088	0.846	0.265	10.900	0.203	0.000	0.000	0.000	37.302
191	26.071	0.924	0.259	9.374	0.190	0.000	0.000	0.000	36.818
193	26.021	0.937	0.262	8.730	0.184	0.000	0.000	0.000	36.136
195	25.729	0.919	0.253	9.223	0.166	0.000	0.000	0.000	36.289
197	24.813	0.887	0.244	10.314	0.155	0.000	0.000	0.000	36.412
199	22.400	0.786	0.233	10.277	0.141	0.000	0.000	0.000	33.837
201	24.423	0.929	0.258	10.193	0.157	0.000	0.000	0.000	35.960
203	24.112	0.970	0.267	10.446	0.168	0.000	0.000	0.000	35.962
205	23.596	0.959	0.274	10.741	0.174	0.000	0.000	0.000	35.744
207	22.462	0.885	0.270	11.673	0.175	0.000	0.000	0.000	35.466

Table N-6. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse countercurrent Fermentation MF (marine inocula, ammonium bicarbonate buffer, LRT = 131.35 day, and VSLR = 8.96 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	12.177	0.546	0.141	9.979	0.115	0.000	0.000	0.000	22.957
2	14.841	0.638	0.185	10.478	0.125	0.000	0.000	0.000	26.267
4	17.319	0.900	0.217	10.814	0.147	0.000	0.000	0.000	29.398
8	21.303	1.159	0.247	10.467	0.165	0.000	0.000	0.000	33.341
10	21.429	1.124	0.252	10.195	0.149	0.000	0.000	0.000	33.150
12	22.566	1.117	0.260	10.043	0.152	0.000	0.000	0.000	34.137
14	26.552	1.149	0.254	10.559	0.146	0.000	0.000	0.000	38.660
16	28.676	1.174	0.249	10.684	0.162	0.000	0.000	0.000	40.945
18	29.212	1.172	0.242	10.064	0.155	0.000	0.000	0.000	40.845
20	32.794	1.211	0.256	9.950	0.242	0.070	0.000	0.000	44.523
22	34.254	1.226	0.266	9.788	0.175	0.000	0.000	0.000	45.709
24	37.416	1.262	0.290	9.697	0.176	0.000	0.000	0.000	48.840
26	37.124	1.222	0.297	8.975	0.196	0.000	0.000	0.000	47.814
30	42.778	1.321	0.314	8.717	0.206	0.000	0.000	0.000	53.335
32	40.082	1.230	0.291	7.881	0.213	0.000	0.000	0.000	49.697
34	43.875	1.326	0.309	8.318	0.241	0.000	0.000	0.000	54.069
36	43.446	1.275	0.306	8.234	0.173	0.000	0.000	0.109	53.543
38	41.433	1.160	0.268	7.150	0.158	0.000	0.000	0.129	50.298
40	45.769	1.286	0.294	7.654	0.183	0.000	0.000	0.173	55.359
42	45.335	1.173	0.268	6.702	0.170	0.000	0.000	0.129	53.778
44	44.835	1.212	0.279	6.617	0.171	0.000	0.000	0.157	53.271
48	45.995	1.285	0.266	5.989	0.173	0.000	0.000	0.173	53.88
50	49.999	1.388	0.274	6.162	0.174	0.000	0.000	0.178	58.175
54	50.172	1.364	0.262	5.378	0.176	0.000	0.000	0.163	57.515
56	50.054	1.321	0.252	5.038	0.169	0.000	0.000	0.163	56.997
58	45.950	1.203	0.224	4.555	0.151	0.000	0.000	0.155	52.239
60	51.730	1.266	0.233	4.682	0.163	0.000	0.000	0.146	58.219
62	48.381	1.200	0.222	4.303	0.150	0.000	0.000	0.130	54.386
66	50.095	1.198	0.215	4.183	0.154	0.000	0.000	0.136	55.981
70	49.876	1.198	0.209	3.921	0.143	0.000	0.000	0.132	55.478
74	50.883	1.227	0.204	3.877	0.142	0.000	0.000	0.138	56.471
78	54.354	1.266	0.203	3.928	0.142	0.000	0.000	0.000	59.894
80	53.533	1.242	0.197	3.903	0.148	0.000	0.000	0.147	59.171
82	48.273	1.132	0.181	3.461	0.129	0.000	0.000	0.148	53.324
98	54.824	1.357	0.194	3.861	0.141	0.000	0.000	0.151	60.529
102	54.455	1.453	0.199	3.969	0.148	0.000	0.000	0.154	60.378

Table N-7. Carboxylic acid concentration (g/L) for hot-lime-water-treated bagasse countercurrent Fermentation MG (marine inocula, ammonium bicarbonate buffer, LRT = 44.72 day, and VSLR = 6.79 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
106	57.672	1.533	0.197	4.042	0.148	0.000	0.000	0.144	63.736
108	49.753	1.386	0.179	3.794	0.131	0.000	0.000	0.134	55.377
110	49.649	1.435	0.187	3.954	0.139	0.000	0.000	0.125	55.488
112	50.997	1.480	0.189	4.149	0.152	0.000	0.000	0.138	57.105
114	47.627	1.379	0.180	3.719	0.138	0.000	0.000	0.123	53.165
116	49.674	1.458	0.190	4.297	0.155	0.000	0.000	0.127	55.9
118	51.859	1.504	0.197	4.463	0.175	0.000	0.000	0.139	58.338
120	50.649	1.479	0.193	4.504	0.169	0.000	0.000	0.134	57.129
122	49.483	1.446	0.187	4.538	0.157	0.000	0.000	0.112	55.921
124	48.856	1.422	0.188	4.357	0.150	0.000	0.000	0.107	55.08
126	51.142	1.467	0.201	4.525	0.160	0.000	0.000	0.121	57.616
128	50.921	1.405	0.207	4.347	0.167	0.000	0.000	0.125	57.172
132	51.391	1.435	0.214	4.124	0.160	0.000	0.000	0.125	57.449
134	50.398	1.457	0.218	3.925	0.157	0.000	0.000	0.119	56.273
136	52.279	1.557	0.230	4.033	0.174	0.000	0.000	0.118	58.391
138	50.778	1.487	0.226	3.729	0.150	0.000	0.000	0.112	56.482
140	51.403	1.484	0.225	3.669	0.147	0.000	0.000	0.103	57.03
142	52.116	1.514	0.221	3.628	0.144	0.000	0.000	0.123	57.746
144	50.673	1.478	0.206	3.431	0.136	0.000	0.000	0.111	56.035
146	53.117	1.522	0.204	3.451	0.135	0.000	0.000	0.138	58.567
148	49.965	1.490	0.194	3.349	0.132	0.000	0.000	0.115	55.245
150	49.917	1.519	0.192	3.383	0.130	0.000	0.000	0.114	55.254
152	50.143	1.470	0.183	3.283	0.124	0.000	0.000	0.109	55.311
154	49.096	1.448	0.188	3.197	0.131	0.000	0.000	0.105	54.166
156	49.344	1.438	0.190	3.183	0.154	0.000	0.000	0.149	54.458
158	51.570	1.517	0.186	3.329	0.129	0.000	0.000	0.118	56.85
160	50.404	1.450	0.174	3.144	0.122	0.000	0.000	0.134	55.428
162	50.748	1.464	0.178	3.203	0.135	0.000	0.000	0.154	55.882
164	51.211	1.409	0.174	3.105	0.128	0.000	0.000	0.119	56.145
166	50.554	1.436	0.184	3.286	0.133	0.000	0.000	0.125	55.718
168	49.979	1.416	0.182	3.155	0.125	0.000	0.000	0.000	54.855

APPENDIX O

CARBOXYLIC ACID PRODUCTION DATA FOR AMMONIA-TREATED BAGASSE COUNTERCURRENT FERMENTATIONS BUFFERED BY AMMONIUM BICARBONATE

Table O-1. Carboxylic acid concentration (g/L) for ammonia-treated bagasse countercurrent Fermentation MH (marine inocula, ammonium bicarbonate buffer, LRT = 55.48 day, and VSLR = 5.74 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	0.967	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.967
2	3.051	0.077	0.000	1.302	0.000	0.000	0.000	0.000	4.430
7	9.747	1.092	0.099	1.522	0.093	0.000	0.000	0.000	12.553
9	12.486	1.508	0.133	1.676	0.140	0.000	0.000	0.000	15.943
11	14.029	1.578	0.151	1.722	0.161	0.000	0.000	0.000	17.641
13	14.572	1.506	0.165	1.731	0.172	0.000	0.000	0.000	18.146
15	16.851	1.500	0.183	1.798	0.183	0.000	0.000	0.057	20.572
17	19.757	1.516	0.203	1.886	0.192	0.000	0.000	0.156	23.710
19	21.245	1.428	0.209	1.904	0.195	0.000	0.000	0.000	24.981
21	23.155	1.298	0.215	1.903	0.184	0.000	0.000	0.000	26.755
23	25.335	1.524	0.218	1.730	0.104	0.000	0.000	0.000	28.912
25	30.365	1.833	0.272	2.206	0.144	0.000	0.000	0.000	34.819
27	32.673	1.742	0.310	2.211	0.180	0.000	0.000	0.000	37.117
31	36.809	1.656	0.331	2.223	0.205	0.000	0.000	0.000	41.224
35	35.021	1.554	0.309	2.019	0.191	0.000	0.000	0.000	39.094
37	35.980	1.544	0.303	2.009	0.195	0.000	0.000	0.000	40.031
39	36.879	1.574	0.306	2.192	0.190	0.000	0.000	0.000	41.140
41	37.297	1.792	0.296	2.271	0.170	0.000	0.000	0.000	41.826
43	37.386	1.811	0.296	2.178	0.169	0.000	0.000	0.000	41.839
45	36.931	1.754	0.287	2.097	0.171	0.000	0.000	0.000	41.240
47	36.585	1.682	0.273	1.991	0.157	0.000	0.000	0.000	40.687
49	35.603	1.592	0.273	1.950	0.169	0.000	0.000	0.000	39.586
51	36.121	1.507	0.262	1.850	0.153	0.000	0.000	0.000	39.893
53	32.006	1.243	0.222	1.551	0.133	0.000	0.000	0.000	35.155
55	35.797	1.420	0.251	1.791	0.144	0.000	0.000	0.000	39.402
57	35.276	1.362	0.233	1.724	0.132	0.000	0.000	0.000	38.727
59	37.375	1.383	0.235	1.718	0.137	0.000	0.000	0.000	40.848

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
61	35.006	1.329	0.232	1.704	0.129	0.000	0.000	0.000	38.400
65	37.620	1.334	0.249	1.848	0.136	0.000	0.000	0.000	41.187
67	39.755	1.414	0.257	1.921	0.136	0.000	0.000	0.000	43.483
69	39.990	2.158	0.257	1.921	0.131	0.000	0.000	0.000	44.457
71	40.167	1.916	0.246	1.807	0.124	0.000	0.000	0.000	44.260
75	39.460	1.641	0.233	1.697	0.117	0.000	0.000	0.000	43.149
77	36.508	1.448	0.224	1.565	0.117	0.000	0.000	0.000	39.862
79	39.047	1.468	0.234	1.579	0.125	0.000	0.000	0.000	42.454
81	39.027	1.481	0.228	1.733	0.116	0.000	0.000	0.000	42.586
83	42.964	1.489	0.226	1.590	0.110	0.000	0.000	0.000	46.380
85	42.509	1.488	0.225	1.670	0.110	0.000	0.000	0.000	46.002
87	40.005	1.403	0.217	1.621	0.000	0.000	0.000	0.119	43.365
89	42.402	1.515	0.220	1.698	0.000	0.000	0.000	0.000	45.836
91	40.301	1.471	0.207	1.652	0.000	0.000	0.000	0.000	43.631
93	36.112	1.280	0.183	1.536	0.000	0.000	0.000	0.000	39.111
95	41.676	1.437	0.191	1.775	0.000	0.000	0.000	0.000	45.079
97	40.813	1.431	0.177	1.728	0.000	0.000	0.000	0.000	44.149
99	41.703	1.435	0.170	1.761	0.000	0.000	0.000	0.141	45.209

Table O-2. Carboxylic acid concentration (g/L) for ammonia-treated bagasse countercurrent Fermentation MK (marine inocula, ammonium bicarbonate buffer, LRT = 30.63 day, and VSLR = 4.42 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	0.899	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.899
2	4.098	0.000	0.000	0.283	0.000	0.000	0.000	0.000	4.381
7	10.527	0.711	0.123	2.320	0.103	0.000	0.000	0.000	13.785
9	13.152	0.857	0.216	2.563	0.145	0.000	0.000	0.045	16.978
13	17.480	0.996	0.361	3.007	0.279	0.000	0.000	0.000	22.123
15	16.560	0.977	0.322	2.942	0.243	0.000	0.000	0.000	21.044
17	18.872	0.990	0.395	3.129	0.309	0.000	0.000	0.000	23.694
19	20.533	0.993	0.422	3.124	0.326	0.000	0.000	0.000	25.398
21	20.806	1.323	0.409	2.783	0.305	0.000	0.000	0.000	25.626
23	22.522	1.393	0.384	2.830	0.278	0.000	0.000	0.000	27.407
25	25.581	1.460	0.414	3.823	0.309	0.000	0.000	0.000	31.588
27	27.694	1.507	0.472	3.434	0.363	0.000	0.000	0.000	33.471
31	30.439	1.560	0.489	3.302	0.381	0.000	0.000	0.000	36.171
33	30.404	1.474	0.456	3.045	0.368	0.000	0.000	0.000	35.747
35	29.508	1.344	0.433	2.874	0.356	0.000	0.000	0.000	34.516
37	28.382	1.303	0.414	2.634	0.334	0.000	0.000	0.000	33.066
39	28.384	1.134	0.380	2.478	0.303	0.000	0.000	0.000	32.678
41	29.918	1.229	0.399	2.674	0.284	0.000	0.000	0.000	34.504
43	29.314	1.118	0.382	2.721	0.273	0.000	0.000	0.000	33.809
45	21.937	0.887	0.298	5.866	0.209	0.000	0.000	0.000	29.196
47	24.695	1.011	0.345	5.882	0.244	0.000	0.000	0.000	32.179
49	24.010	1.201	0.329	6.502	0.224	0.000	0.000	0.000	32.266
51	23.033	1.113	0.309	7.077	0.214	0.000	0.000	0.000	31.746
53	23.829	1.122	0.295	6.746	0.203	0.000	0.000	0.000	32.195
55	24.446	1.169	0.291	5.365	0.214	0.000	0.000	0.000	31.485
57	24.302	1.211	0.278	6.399	0.207	0.000	0.000	0.000	32.397
59	25.062	1.173	0.261	5.997	0.199	0.000	0.000	0.000	32.692
61	26.426	1.175	0.269	4.979	0.199	0.000	0.000	0.000	33.048
65	28.512	1.114	0.288	4.400	0.214	0.000	0.000	0.000	34.528
65	29.758	1.173	0.294	3.919	0.215	0.000	0.000	0.000	35.359
67	30.129	1.130	0.299	3.564	0.212	0.000	0.000	0.000	35.334
71	29.803	1.094	0.276	2.972	0.193	0.000	0.000	0.000	34.338
73	28.868	0.962	0.264	2.839	0.188	0.000	0.000	0.000	33.121
75	28.607	0.967	0.255	2.789	0.184	0.000	0.000	0.000	32.803
77	28.985	1.094	0.250	2.920	0.180	0.000	0.000	0.000	33.430
79	29.658	0.997	0.248	2.743	0.172	0.000	0.000	0.000	33.818

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
81	30.039	1.112	0.249	2.773	0.167	0.000	0.000	0.000	34.339
83	33.380	1.170	0.254	2.811	0.162	0.000	0.000	0.000	37.777
85	30.604	1.122	0.245	2.700	0.160	0.000	0.000	0.000	34.830
87	29.602	1.082	0.248	2.669	0.153	0.000	0.000	0.000	33.755
89	30.579	1.168	0.265	2.774	0.167	0.000	0.000	0.000	34.952
91	30.592	1.108	0.255	2.744	0.154	0.000	0.000	0.000	34.853
93	30.662	1.100	0.241	2.727	0.141	0.000	0.000	0.000	34.871
95	31.494	1.063	0.250	2.815	0.148	0.000	0.000	0.000	35.770
97	32.649	1.018	0.267	2.875	0.160	0.000	0.000	0.000	36.969
99	33.564	0.990	0.243	2.719	0.151	0.000	0.000	0.000	37.667

Table O-3. Carboxylic acid concentration (g/L) for ammonia-treated bagasse countercurrent Fermentation ML (marine inocula, ammonium bicarbonate buffer, LRT = 26.22 day, and VSLR = 3.07 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	1.079	0.000	0.000	0.064	0.000	0.000	0.000	0.000	1.143
2	2.475	0.000	0.000	1.636	0.000	0.000	0.000	0.000	4.111
7	21.495	0.325	0.105	0.766	0.070	0.000	0.000	0.000	22.762
9	26.097	0.383	0.100	0.857	0.000	0.000	0.000	0.000	27.437
11	20.512	1.269	0.163	0.846	0.114	0.000	0.000	0.000	22.904
13	27.420	0.437	0.067	0.975	0.000	0.000	0.000	0.000	28.899
15	29.626	0.448	0.061	1.029	0.000	0.000	0.000	0.000	31.165
17	30.474	0.499	0.074	1.138	0.000	0.000	0.000	0.047	32.232
19	23.165	0.916	0.157	1.274	0.081	0.000	0.000	0.000	25.593
21	24.573	0.941	0.164	1.472	0.062	0.000	0.000	0.000	27.213
23	20.225	0.789	0.147	1.324	0.000	0.000	0.000	0.000	22.485
25	28.137	1.119	0.224	2.001	0.104	0.000	0.000	0.000	31.586
27	30.212	1.217	0.227	2.032	0.117	0.000	0.000	0.000	33.805
31	34.258	1.655	0.250	2.086	0.141	0.000	0.000	0.000	38.390
33	34.873	1.589	0.260	2.049	0.159	0.000	0.000	0.000	38.931
35	35.424	1.503	0.273	2.050	0.181	0.000	0.000	0.000	39.430
37	35.888	1.362	0.276	1.998	0.193	0.000	0.000	0.000	39.717
39	33.837	1.224	0.276	1.938	0.194	0.000	0.000	0.000	37.469
41	35.158	1.477	0.303	2.147	0.219	0.000	0.000	0.000	39.304
43	33.001	1.298	0.294	2.113	0.212	0.000	0.000	0.000	36.917
45	28.301	1.034	0.266	2.096	0.189	0.000	0.000	0.000	31.887
47	27.188	1.078	0.275	2.317	0.197	0.000	0.000	0.000	31.055
49	25.347	0.898	0.273	2.348	0.197	0.000	0.000	0.000	29.063
51	22.908	0.883	0.267	4.820	0.187	0.000	0.000	0.000	29.065
53	21.226	0.774	0.000	5.187	0.174	0.000	0.000	0.000	27.362
55	20.264	0.680	0.000	4.886	0.166	0.000	0.000	0.000	25.996
57	20.844	0.680	0.252	5.485	0.166	0.000	0.000	0.000	27.427
59	19.990	0.571	0.000	5.591	0.165	0.000	0.000	0.000	26.317
61	18.705	0.497	0.241	5.714	0.156	0.000	0.000	0.000	25.313
65	21.698	0.591	0.292	4.441	0.210	0.000	0.000	0.000	27.233
67	21.997	0.600	0.309	4.365	0.208	0.000	0.000	0.000	27.479
69	21.548	0.605	0.322	4.575	0.216	0.000	0.000	0.000	27.266
73	20.864	0.537	0.328	4.372	0.197	0.000	0.000	0.000	26.298
75	21.897	0.613	0.327	4.429	0.198	0.000	0.000	0.000	27.463
77	22.741	0.641	0.340	4.437	0.197	0.000	0.000	0.000	28.355

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
79	22.167	0.600	0.337	4.333	0.186	0.000	0.000	0.000	27.623
81	22.919	0.648	0.353	4.476	0.198	0.000	0.000	0.000	28.593
83	26.757	0.726	0.000	4.338	0.224	0.000	0.000	0.000	32.046
85	24.709	0.696	0.000	4.119	0.210	0.000	0.000	0.000	29.734
87	23.966	0.707	0.375	3.986	0.210	0.000	0.000	0.000	29.244
89	25.467	0.778	0.398	4.098	0.230	0.000	0.000	0.000	30.971
91	24.787	0.730	0.405	3.431	0.232	0.000	0.000	0.000	29.585
93	25.003	0.757	0.400	2.889	0.227	0.000	0.000	0.000	29.276
95	25.540	0.767	0.392	2.895	0.203	0.000	0.000	0.000	29.797
97	26.681	0.794	0.395	2.675	0.195	0.000	0.000	0.000	30.741
99	26.446	0.775	0.362	2.380	0.167	0.000	0.000	0.000	30.131

Table O-4. Carboxylic acid concentration (g/L) for ammonia-treated bagasse countercurrent Fermentation NH (marine inocula, ammonium bicarbonate buffer, LRT = 45.18 day, and VSLR = 5.30 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
101	41.963	1.474	0.164	1.783	0.000	0.000	0.000	0.000	45.384
103	41.316	1.602	0.153	1.721	0.000	0.000	0.000	0.000	44.792
105	43.312	1.583	0.153	1.845	0.000	0.000	0.000	0.000	46.893
109	41.427	1.648	0.145	1.703	0.000	0.000	0.000	0.000	44.924
113	42.047	1.540	0.146	1.723	0.000	0.000	0.000	0.000	45.456
115	42.667	1.593	0.137	1.701	0.000	0.000	0.000	0.000	46.098
117	38.781	1.454	0.129	1.607	0.000	0.000	0.000	0.000	41.972
119	40.908	1.514	0.150	1.807	0.000	0.000	0.000	0.000	44.379
121	40.425	1.495	0.144	1.839	0.000	0.000	0.000	0.000	43.903
123	41.636	1.652	0.156	1.969	0.000	0.000	0.000	0.000	45.413
125	42.147	1.609	0.153	1.938	0.000	0.000	0.000	0.000	45.848
127	42.756	1.820	0.159	2.017	0.000	0.000	0.000	0.000	46.753
129	41.472	1.617	0.142	2.004	0.000	0.000	0.000	0.000	45.235
131	40.409	1.480	0.151	2.022	0.000	0.000	0.000	0.000	44.062
133	38.853	1.459	0.152	2.053	0.000	0.000	0.000	0.000	42.516
135	38.574	1.372	0.139	2.192	0.000	0.000	0.000	0.000	42.277
137	40.306	1.482	0.143	2.269	0.000	0.000	0.000	0.000	44.200
139	39.695	1.393	0.159	2.345	0.000	0.000	0.000	0.000	43.593
141	41.117	1.684	0.169	2.464	0.000	0.000	0.000	0.000	45.434
143	40.980	1.597	0.169	2.596	0.000	0.000	0.000	0.000	45.342
145	41.396	1.540	0.179	2.559	0.000	0.000	0.000	0.000	45.674
147	39.957	1.412	0.190	2.473	0.000	0.000	0.000	0.000	44.033
149	38.724	1.362	0.206	2.464	0.000	0.000	0.000	0.000	42.756
151	39.458	1.567	0.194	2.443	0.000	0.000	0.000	0.000	43.663
153	38.572	1.403	0.201	2.480	0.000	0.000	0.000	0.000	42.655
161	38.212	1.606	0.000	2.477	0.000	0.000	0.000	0.000	42.295
163	39.371	1.600	0.155	2.484	0.000	0.000	0.000	0.000	43.611

Table O-5. Carboxylic acid concentration (g/L) for ammonia-treated bagasse countercurrent Fermentation NK (marine inocula, ammonium bicarbonate buffer, LRT = 32.85 day, and VSLR = 4.19 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
101	32.773	0.945	0.221	2.449	0.149	0.000	0.000	0.000	36.538
103	34.020	1.001	0.201	2.281	0.146	0.000	0.000	0.000	37.649
105	34.870	0.953	0.195	2.127	0.149	0.000	0.000	0.000	38.295
109	35.550	1.063	0.159	1.786	0.128	0.000	0.000	0.000	38.686
113	34.563	0.946	0.184	1.616	0.120	0.000	0.000	0.000	37.428
115	35.481	0.898	0.131	1.497	0.129	0.000	0.000	0.000	38.137
117	33.549	0.839	0.150	1.455	0.121	0.000	0.000	0.000	36.114
119	32.812	0.873	0.150	1.482	0.123	0.000	0.000	0.000	35.441
121	32.053	0.914	0.137	1.476	0.115	0.000	0.000	0.000	34.695
123	33.385	0.982	0.153	1.649	0.122	0.000	0.000	0.000	36.292
125	30.953	0.900	0.131	1.579	0.132	0.000	0.000	0.000	33.695
127	32.363	0.868	0.132	1.595	0.101	0.000	0.000	0.000	35.060
129	33.794	1.254	0.172	1.738	0.123	0.000	0.000	0.000	37.082
131	34.573	1.187	0.190	1.847	0.121	0.000	0.000	0.000	37.918
133	33.184	1.109	0.177	1.861	0.117	0.000	0.000	0.000	36.449
135	33.159	1.098	0.000	1.988	0.116	0.000	0.000	0.000	36.361
137	32.939	1.017	0.166	1.941	0.120	0.000	0.000	0.000	36.183
139	30.831	0.904	0.000	1.866	0.125	0.000	0.000	0.000	33.726
141	33.184	0.888	0.204	1.848	0.117	0.000	0.000	0.000	36.240
143	34.772	1.314	0.197	1.913	0.110	0.000	0.000	0.000	38.306
145	33.606	1.235	0.200	1.881	0.000	0.000	0.000	0.000	36.922
147	33.673	1.203	0.191	1.893	0.000	0.000	0.000	0.000	36.960
149	32.635	1.143	0.198	2.032	0.000	0.000	0.000	0.000	36.007
151	34.140	1.378	0.231	2.310	0.000	0.000	0.000	0.000	38.059
153	33.310	1.194	0.220	2.350	0.000	0.000	0.000	0.000	37.075
157	34.345	1.166	0.174	2.331	0.000	0.000	0.000	0.000	38.017
161	32.128	1.023	0.146	2.287	0.106	0.000	0.000	0.000	35.691

Table O-6. Carboxylic acid concentration (g/L) for ammonia-treated bagasse countercurrent Fermentation NL (marine inocula, ammonium bicarbonate buffer, LRT = 29.94 day, and VSLR = 2.74 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
101	27.285	0.763	0.000	2.307	0.157	0.000	0.000	0.000	30.512
103	27.636	0.791	0.000	2.248	0.154	0.000	0.000	0.000	30.828
105	28.375	0.775	0.314	2.075	0.149	0.000	0.000	0.000	31.689
109	26.330	0.771	0.301	1.700	0.167	0.000	0.000	0.000	29.269
111	25.097	0.706	0.000	1.472	0.142	0.000	0.000	0.000	27.417
113	25.947	0.731	0.205	1.558	0.148	0.000	0.000	0.000	28.589
115	26.159	0.719	0.215	1.431	0.155	0.000	0.000	0.000	28.680
117	26.497	0.699	0.172	1.400	0.139	0.000	0.000	0.000	28.907
119	27.293	0.744	0.131	1.461	0.121	0.000	0.000	0.000	29.750
121	25.642	0.725	0.166	1.329	0.131	0.000	0.000	0.000	27.992
123	26.703	0.734	0.165	1.306	0.111	0.000	0.000	0.000	29.019
125	27.411	0.768	0.168	1.564	0.111	0.000	0.000	0.000	30.022
127	25.980	0.732	0.190	1.846	0.000	0.000	0.000	0.000	28.748
129	29.481	0.796	0.230	1.867	0.111	0.000	0.000	0.000	32.484
131	27.025	0.695	0.220	1.758	0.000	0.000	0.000	0.000	29.698
133	26.565	0.650	0.211	1.558	0.000	0.000	0.000	0.000	28.984
135	27.512	0.708	0.000	1.572	0.000	0.000	0.000	0.000	29.792
137	28.535	0.753	0.000	1.574	0.107	0.000	0.000	0.000	30.968
139	26.454	0.739	0.000	1.410	0.000	0.000	0.000	0.000	28.603
141	27.933	0.791	0.231	1.451	0.000	0.000	0.000	0.000	30.406
143	27.403	0.761	0.000	1.449	0.000	0.000	0.000	0.000	29.613
147	26.808	0.720	0.210	1.470	0.000	0.000	0.000	0.000	29.208
149	26.550	0.740	0.198	1.571	0.117	0.000	0.000	0.000	29.176
151	25.128	0.705	0.179	1.515	0.123	0.000	0.000	0.000	27.650
153	24.864	0.708	0.163	1.646	0.116	0.000	0.000	0.000	27.496
157	24.075	0.731	0.177	1.782	0.121	0.000	0.000	0.000	26.886
161	26.019	0.934	0.266	2.008	0.176	0.000	0.000	0.000	29.403

APPENDIX P

CARBOXYLIC ACID PRODUCTION DATA FOR AIR-LIME-TREATED BAGASSE COUNTERCURRENT FERMENTATIONS BUFFERED BY AMMONIUM BICARBONATE

Table P-1. Carboxylic acid concentration (g/L) for air-lime-treated bagasse countercurrent Fermentation TA (marine inocula, ammonium bicarbonate buffer, LRT = 31.95 day, and VSLR = 4.83 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
0	1.944	0.000	0.000	0.108	0.000	0.000	0.000	0.000	2.052
2	4.167	0.140	0.000	1.590	0.000	0.000	0.000	0.000	5.897
4	7.107	0.194	0.000	1.694	0.055	0.000	0.000	0.000	9.050
8	9.834	0.273	0.143	1.616	0.108	0.000	0.000	0.000	11.974
14	16.320	0.551	0.212	2.022	0.168	0.000	0.067	0.000	19.341
20	25.698	0.822	0.309	2.444	0.215	0.000	0.071	0.000	29.558
24	25.228	0.948	0.356	3.677	0.227	0.000	0.000	0.000	30.436
26	26.169	0.907	0.394	3.820	0.271	0.000	0.000	0.000	31.560
26	25.414	0.932	0.363	3.676	0.243	0.000	0.000	0.000	30.628
28	22.918	0.831	0.332	3.337	0.229	0.000	0.000	0.000	27.646
32	26.079	0.898	0.412	3.860	0.276	0.000	0.000	0.000	31.525
34	26.501	0.897	0.420	3.941	0.259	0.000	0.000	0.000	32.018
36	25.275	0.789	0.377	4.341	0.240	0.000	0.000	0.000	31.022
38	26.965	0.748	0.383	5.110	0.263	0.000	0.000	0.000	33.468
40	27.755	0.785	0.440	5.169	0.304	0.000	0.000	0.000	34.454
42	27.375	0.831	0.000	7.845	0.318	0.000	0.000	0.000	36.370
44	24.921	1.082	0.395	8.348	0.305	0.000	0.000	0.000	35.051
46	22.861	0.862	0.325	7.469	0.237	0.000	0.000	0.000	31.754
48	23.829	1.126	0.295	6.579	0.203	0.000	0.000	0.000	32.031
51	26.608	1.402	0.319	6.011	0.246	0.000	0.000	0.000	34.586
53	29.002	1.596	0.356	5.489	0.287	0.000	0.000	0.000	36.730
55	29.279	1.590	0.354	5.322	0.316	0.000	0.000	0.000	36.861
57	28.158	1.483	0.353	5.220	0.318	0.000	0.000	0.000	35.532
59	30.246	1.391	0.380	5.191	0.307	0.000	0.000	0.000	37.515
61	30.946	1.371	0.398	5.406	0.321	0.000	0.000	0.000	38.443
63	31.901	1.436	0.402	5.456	0.316	0.000	0.000	0.000	39.511
65	33.278	1.438	0.405	5.402	0.321	0.000	0.000	0.000	40.843

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
67	30.732	1.376	0.399	5.111	0.324	0.000	0.000	0.000	37.942
71	34.140	1.563	0.409	5.295	0.290	0.000	0.000	0.000	41.696
73	35.607	1.568	0.420	5.332	0.271	0.000	0.000	0.000	43.198
73	35.748	1.518	0.406	5.050	0.271	0.000	0.000	0.000	42.993
75	36.235	1.591	0.427	5.204	0.262	0.000	0.000	0.000	43.719
75	36.371	1.542	0.412	4.929	0.259	0.000	0.000	0.000	43.513
77	35.878	1.546	0.409	4.859	0.240	0.000	0.000	0.000	42.932
79	36.850	1.550	0.426	4.881	0.238	0.000	0.000	0.000	43.944
81	38.224	1.589	0.434	5.034	0.231	0.000	0.000	0.000	45.513
83	38.990	1.565	0.451	4.963	0.236	0.000	0.000	0.000	46.205
85	40.741	1.518	0.470	5.069	0.239	0.000	0.000	0.000	48.037
87	39.012	1.362	0.438	4.930	0.195	0.000	0.000	0.000	45.938
89	36.888	1.325	0.426	5.155	0.168	0.000	0.000	0.000	43.963
95	38.145	1.239	0.386	4.810	0.147	0.000	0.000	0.000	44.728
97	40.216	1.280	0.370	5.120	0.150	0.000	0.000	0.000	47.136
99	40.632	1.263	0.340	4.700	0.143	0.000	0.000	0.000	47.078
101	39.964	1.253	0.347	4.790	0.144	0.000	0.000	0.000	46.498
103	31.923	1.042	0.270	3.852	0.112	0.000	0.000	0.000	37.198
105	37.150	1.189	0.296	4.324	0.132	0.000	0.000	0.000	43.092
107	36.483	1.153	0.280	4.179	0.129	0.000	0.000	0.000	42.223
109	38.106	1.214	0.000	4.280	0.124	0.000	0.000	0.000	43.724
109	36.350	1.161	0.240	4.104	0.128	0.000	0.000	0.000	41.982
111	33.433	1.049	0.312	3.111	0.269	0.000	0.000	0.000	38.173
113	33.573	1.063	0.215	3.625	0.117	0.000	0.000	0.000	38.593
123	36.897	1.077	0.157	3.181	0.139	0.000	0.000	0.000	41.450
124	35.834	1.069	0.153	3.013	0.131	0.000	0.000	0.000	40.199
127	35.328	1.108	0.141	3.023	0.130	0.000	0.000	0.000	39.729

Table P-2. Carboxylic acid concentration (g/L) for air-lime-treated bagasse countercurrent Fermentation TB (marine inocula, ammonium bicarbonate buffer, LRT = 25.23 day, and VSLR = 4.05 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
20	12.206	0.450	0.130	2.696	0.151	0.000	0.000	0.000	15.634
22	15.694	0.542	0.174	3.487	0.186	0.000	0.000	0.000	20.083
24	15.822	0.523	0.188	4.022	0.175	0.000	0.000	0.000	20.730
28	19.857	0.679	0.225	4.824	0.200	0.000	0.000	0.000	25.784
34	30.980	1.026	0.315	4.555	0.222	0.000	0.000	0.000	37.097
36	34.798	1.152	0.342	4.690	0.238	0.000	0.000	0.000	41.220
38	38.791	1.257	0.368	4.531	0.238	0.000	0.000	0.000	45.185
40	39.472	1.290	0.389	4.594	0.256	0.000	0.000	0.000	46.002
42	41.019	1.333	0.406	4.595	0.278	0.000	0.000	0.000	47.632
46	39.993	1.333	0.427	4.469	0.310	0.000	0.000	0.000	46.532
52	41.402	1.287	0.424	3.900	0.295	0.000	0.000	0.000	47.309
54	40.127	1.255	0.409	3.706	0.284	0.000	0.000	0.000	45.781
56	41.219	1.307	0.412	3.573	0.296	0.000	0.000	0.000	46.807
58	40.123	1.291	0.399	3.478	0.291	0.000	0.000	0.000	45.582
60	34.010	1.144	0.000	3.188	0.276	0.000	0.000	0.000	38.617
62	32.261	1.025	0.317	2.872	0.258	0.000	0.000	0.000	36.733
64	33.585	1.045	0.321	2.977	0.263	0.000	0.000	0.000	38.191
66	26.679	1.067	0.285	3.921	0.193	0.000	0.000	0.000	32.144
70	29.705	0.898	0.277	2.693	0.225	0.000	0.000	0.000	33.797
72	27.338	0.829	0.252	2.526	0.194	0.000	0.000	0.000	31.139
80	28.579	1.044	0.217	2.577	0.147	0.000	0.000	0.000	32.565
81	29.935	1.030	0.221	2.589	0.148	0.000	0.000	0.000	33.923
84	30.117	1.031	0.214	2.536	0.141	0.000	0.000	0.000	34.039
86	30.018	1.114	0.193	2.597	0.125	0.000	0.000	0.000	34.047
88	29.017	0.993	0.183	2.506	0.120	0.000	0.000	0.000	32.820
90	30.762	1.048	0.191	2.742	0.126	0.000	0.000	0.000	34.868

Table P-3. Carboxylic acid concentration (g/L) for air-lime-treated bagasse countercurrent Fermentation TC (marine inocula, ammonium bicarbonate buffer, LRT = 23.54 day, and VSLR = 2.58 (g VS/L liquid·day)).

Days	C2	C3	IC4	C4	IC5	C5	C6	C7	Total
20	10.331	0.528	0.000	2.529	0.000	0.000	0.000	0.000	13.388
21	10.752	0.507	0.000	2.355	0.000	0.000	0.000	0.588	14.202
22	14.723	0.618	0.147	2.661	0.131	0.000	0.000	0.000	18.280
24	15.976	0.574	0.171	2.782	0.155	0.000	0.000	0.000	19.657
28	20.190	0.758	0.261	3.532	0.227	0.000	0.000	0.000	24.967
30	22.692	0.859	0.278	3.788	0.238	0.000	0.000	0.000	27.854
32	26.181	1.003	0.323	4.568	0.266	0.000	0.000	0.000	32.341
34	28.278	1.122	0.341	5.013	0.277	0.000	0.000	0.000	35.032
38	32.726	1.366	0.387	6.011	0.294	0.000	0.000	0.000	40.784
40	32.273	1.366	0.393	6.272	0.308	0.000	0.000	0.000	40.611
42	26.729	1.151	0.372	5.435	0.267	0.000	0.000	0.000	33.955
46	31.974	1.331	0.394	5.887	0.273	0.000	0.000	0.000	39.858
52	33.223	1.262	0.000	4.789	0.254	0.000	0.000	0.000	39.528
54	31.595	1.252	0.350	4.372	0.228	0.000	0.000	0.000	37.796
56	32.130	1.238	0.363	4.605	0.248	0.000	0.000	0.000	38.583
58	31.403	1.255	0.323	4.111	0.200	0.000	0.000	0.000	37.292
60	28.168	1.058	0.289	3.793	0.167	0.000	0.000	0.000	33.474
62	25.716	0.954	0.264	3.459	0.159	0.000	0.000	0.000	30.552
64	26.071	0.929	0.275	3.825	0.170	0.000	0.000	0.000	31.269
66	23.577	0.964	0.214	2.263	0.176	0.000	0.000	0.000	27.195
68	30.669	0.960	0.183	2.696	0.148	0.000	0.000	0.000	34.656
68	30.253	0.953	0.171	2.910	0.150	0.000	0.000	0.000	34.437
70	24.648	0.883	0.242	3.379	0.157	0.000	0.000	0.000	29.311
72	24.583	0.927	0.227	3.220	0.156	0.000	0.000	0.000	29.113
80	23.934	0.777	0.197	2.237	0.143	0.000	0.000	0.000	27.288
84	24.863	0.804	0.000	2.000	0.130	0.000	0.000	0.000	27.797
88	24.006	0.757	0.151	2.171	0.000	0.000	0.000	0.000	27.085
90	26.633	0.854	0.000	2.450	0.107	0.000	0.000	0.000	30.043
94	27.000	0.899	0.171	2.659	0.118	0.000	0.000	0.000	30.847

VITA

Name:	Zhihong Fu
Address:	C/O Dr. Mark T. Holtzapple
	Department of Chemical Engineering
	Texas A&M University
	College Station, TX, 77843-3122
E-mail:	zhihongfu@hotmail.com
Education:	B.S., Chemical Engineering, Xiamen University, PR China, July 1996
	M.S., Chemical Engineering, Xiamen University, PR China, July 1999
	Ph.D., Chemical Engineering, Texas A&M University, USA, May 2007