PILOT-SCALE FERMENTATION AND LABORATORY NUTRIENT STUDIES ON MIXED-ACID FERMENTATION

A Dissertation

by

AARON DOUGLAS SMITH

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 2011

Major Subject: Chemical Engineering

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Approved by:

Chair of Committee,	Mark T. Holtzapple
Committee Members,	Charles J. Glover
	Sergio Capareda
	Kenneth Hall
Head of Department,	Michael Phisko

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ABSTRACT

Pilot-scale Fermentation and Laboratory Nutrient Studies on Mixed-acid Fermentation. (May 2011)

Aaron Douglas Smith, B.S., Mississippi State University

Chair of Advisory Committee: Dr. Mark T. Holtzapple

Via mixed-culture fermentation, the MixAlco^{TM} produces carboxylic acids, which are chemically converted into industrial chemicals and hydrocarbon fuels.

Using pilot fermentation data, The Continuum Particle Distribution Model (CPDM) overestimated acid concentration (30–90% error) but more closely estimated conversion (<15% error). Incorporating the effect of air into the model reduced the absolute error of all predictions by >50%.

To analyze fermentation data with semi-continuous streams, the Slope method calculates the average flowrate of material from the slope of the moving cumulative sum with respect to time. Although the Slope method does not significantly improve accuracy, it dramatically reduces error compared to traditional techniques (>40% vs. <2%).

Nutrients are essential for microbial growth and metabolism. For a four-bottle fermentation train, five nutrient contacting patterns (single-point nutrient addition to Fermentors F1, F2, F3, F4, and multi-point parallel addition) were investigated. Compared to the traditional nutrient contacting method (all nutrients fed to F1), the near-optimal feeding strategies improved exit yield, culture yield, process yield, exit acetate-equivalent yield, conversion, and total acid productivity by approximately 31%, 39%, 46%, 31%, 100%, and 19%, respectively.

To estimate nitrogen concentration profiles, a segregated-nitrogen model uses separate mass balances for solid- and liquid-phase nitrogen; the nitrogen reaction flux between phases is assumed to be zero. Using five fermentation trains, each with a

different nutrient contacting pattern, the model predictions capture basic behavior; therefore, it is a reasonable tool for estimating and controlling nitrogen profiles.

To determine the optimal scenario for mixed-acid fermentations, an array of batch fermentations was performed that independently varied the C/N ratio and the blend of carbohydrate (office paper) and nutrient (wet chicken manure (CM)). Reactant was defined as non-acid volatile solids (NAVS). C/N ratios were based on non-acid carbon (C_{NA}). A blend of 93% paper and 7% wet CM (dry basis) with a C/N ratio of 37 g C_{NA}/g N had the highest culture yield (0.21 g $acid_{produced}$/g $NAVS_{initial}$), total acid productivity (0.84 g $acid_{produced}/(L_{liq} \cdot d)$), and conversion (0.43 g $NAVS_{consumed}$/g $NAVS_{initial}$).

DEDICATION

This dissertation is dedicated to my first, favorite, and best engineering "professor" – my Dad, Stephen D. Smith. It was from him that I learned to work with my hands, design, build, and think like an engineer. I am forever indebted to his many lessons.

ACKNOWLEDGEMENTS

May all honor and glory go to God! I am forever grateful for the talents and blessings God has given me. My goal is to use my life to make the world a better place. It is my prayer that the results of this research be used to develop renewable chemicals and fuels that benefit society.

I am forever grateful for my parents, Linda L., and Stephen D. Smith, who taught me about God and faith, instilled in me values, morals, and a strong work ethic, gave me life skills, taught me to be a leader, and graciously supported me throughout my education. My parents have been outstanding examples of wisdom, work ethic, responsibility, leadership, love, and Christ-like behavior that have made their lessons all the more powerful. Thank you for "walking the walk." To my mother, I am especially thankful for teaching me a love for food, how to cook, and how to love others. To my father, I am thankful for teaching about science, engineering, how to work with my hands, how to love my wife, and how to be an honest man.

I am thankful for my wife, Megan H. Smith, who has made my life richer, deeper, and more meaningful than I could ever imagine. She has always been a source of unconditional love, support, and patience.

Dr. Holtzapple has been a great source of inspiration, optimism, and opportunity. Because of his faith in me I have had many awesome opportunities as a graduate student; I managed and operated a pilot plant, first-authored two book chapters, conducted original research, and represented Dr. Holtzapple in Beijing, China at the China-US Relations conference. I am very appreciative of Dr. Holtzapple for his valuable counsel, support, enthusiasm, and friendship. A student's attitude about graduate school is largely a reflection of his relationship with his advisor. For me graduate school has been a fun, positive, and rewarding experience that will forever influence my life. Dr. Holtzapple, thank you!

I would like to thank my committee members Dr. Charles Glover, Dr. Kenneth Hall, and Dr. Sergio Capareda for their time, feedback, critiques, and encouragement

that have helped me throughout my graduate career.

Managing and running the pilot plant was a large task that would not have been possible without the help of numerous individuals. To each of the 55+ student workers who helped me over the years, I am thankful for your positive attitude and hard work. A special thanks to outstanding student workers Austin Bond, Michael Dunn, Amber Patek, and Kelley Fowler for your leadership and 110% effort. Randy Merek, Randy Tucker, Louis Muntiz were generous with their time and helped repair, build, and install numerous pilot plant and laboratory items.

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Terrabon, Inc. (Houston, TX) generously funded many of my research projects. To David Carrabba, co-founder of Terrabon, and Gary Luce, CEO of Terrabon, Inc., I am especially thankful for your support and interest in my research. I have learned many “real world” lessons from my interactions with both of you.

NOMENCLATURE

A	mass of carboxylic acid, g
$[A]$	total carboxylic acid concentration, g/$L_{liq.}$
aceq	acetic acid equivalents concentration, g/$L_{liq.}$
ATP	adenosine triphosphate
B_i	Fermentor *i* bottle plus centrifuge cake, g
C	conversion, g NAVS consumed/g NAVS in feed
CM	chicken manure
C/N	carbon-nitrogen ratio, g C_{NA}/g N
C_{NA}	non-acid carbon, g
COD	chemical oxygen demand
CPDM	continuum particle distribution model
D	standard deviation
EIA	Energy Information Agency
F_i	bulk fermentation mass in Fermentor *i*, g wet (as-is)
F*i*	Fermentor *i*
FID	flame ionization detector
FL	filter liquid
FS	filter solids (i.e., cake)
GAC	granular activated carbon
GC	gas chromatograph
GDP	gross domestic product
I	ash content, g ash/g dry sample
IR	infrared
K_{Fi}	the average mass of wet solid cake in Fermentor *i*, g
L	transfer liquid stream flowrate, g wet (as-is)/d (or T)
LRT	liquid retention time, d
LV_{Fi}	liquid volume in Fermentor *i*, $L_{liq.}$

m	slope
M	moisture content, g moisture/g wet (as-is) sample
MRT	moisture retention time, d
MSW	municipal solid waste
n	number of transfers
N	“no”
N	nutrient-rich substrate feedrate, g wet (as-is)/d (or T)
N	number of data points (Section 5 only)
NAVS	non-acid volatile solids, g
NIMBY	“not in my back yard”
NOP	normalized operating parameters
NREL	National Renewable Energy Laboratory
OPEC	Organization of Petroleum Exporting Countries
P	office paper
P	total acid productivity, g acid produced/($L_{liq.}\cdot$d)
PE ratio	process-exit yield ratio
PID	proportional-integral-derivative
PM	percolation method
PVC	polyvinyl chloride
Q	total inlet liquid flowrate, L/d (or T)
S	transfer solid stream flowrate, g wet (as-is)/ d (or T)
S	sum of squares of deviations (Section 5 only)
SC_{Fi}	solid concentration of Fermentor i, g NAVS in Fermentor i/$L_{liq.}$ in Fermentor i
SLM	solid-liquid mixture
SRT	solid retention time, d
SS	steady state
SSE	sum of squared errors
t	time

T	time period between transfers, ~56 h
TAMU	Texas A&M University
TCD	thermal conductivity detector
TKN	total Kjeldahl nitrogen
TLV	total liquid volume in all fermentors, L
US	United States
USB	University Services Building
USD	United States dollar
USDOE	United States Department of Energy
VFA	volatile fatty acids
VS	volatile solids, g
VSLR	volatile solids loading rate, g NAVS/$L_{liq.}$
W_i	solids-retained-plus-bottle-weight set point, g
WAS	waste activated sludge
x_i	x value of *i*-th data point
X	may represent F, S, or L
X_i	x-value data array (Section 5 only)
y_i	y value of *i*-th data point
Y	“yes”
Y_i	y-value data array (Section 5 only)
Y_{ATP}	cell growth yield, g cell dry matter/mol ATP
Y_F	feed yield, g acid in feed/g NAVS in feed
Y_E	exit yield, g acid exiting fermentation/g NAVS in feed
Y_C	culture yield, g acid produced/g NAVS in feed
Y_P	process yield, g acid in product liquid/g NAVS in feed
Z	final value of product and/or quotient

Greek Symbols

α	acetic acid equivalents concentration, mol/$L_{liq.}$
δ_i	error of Value *i*
η	soluble nitrogen mass fraction, g soluble N/g total N
ν	nitrogen content, g N/g wet (as-is) sample
ρ	density, g/mL or lb/gal
σ	total acid selectivity, g acid produced/g NAVS consumed
φ	moles acid/moles aceq

Subscripts

For *M*, *I*, ν, and η, the subscript denotes the corresponding stream or material.

For *F*, *S*, *L*, and [*A*] the subscript denotes the fermentor from which the material or stream came.

For *N*, the subscript denotes the fermentor the nutrient-rich substrate is fed.

Subscript *i* is a number placeholder.

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1. INTRODUCTION

1.1. The energy gap

Population growth and increased quality of life (e.g., motorized transportation, air conditioning, electric devices) increase the global demand for energy (Salameh, 2003). Historically, fossil fuels have supplied this demand. With regard to petroleum-derived liquid fuels (gasoline, diesel), there is growing concern over the projected discrepancy between increased demand and decreased supply (Asif and Muneer, 2007; Salameh, 2003; Smith, 2007). To address this energy crisis, technology must be developed to reduce demand *and* increase supply. Because it is unlikely that population growth and quality of life will be voluntarily decreased, the only practical option for reducing energy demand is to develop energy-efficiency technology. Because of the growing environmental concern over the use of fossil fuels and projected decreased petroleum production, increasing energy supply requires development of non-fossil-fuel-based technology such as nuclear power, renewable electricity (e.g., solar, wind, hydro, geothermal), and renewable transportation fuels (i.e., biofuels) (Asif and Muneer, 2007; Salameh, 2003).

1.2. The need for bioenergy in the United States

In addition to addressing global supply-and-demand concerns, the United States must aggressively develop bioenergy to abate global warming and build energy security.

1.2.1. Global warming

The premise of global warming is that the combustion of fossil fuels (sequestered carbon) increases the concentration of carbon dioxide (greenhouse gas) in the atmosphere, thereby increasing the earth's temperature (Cox et al., 2000; Houghton, 2005; Mitchell et al., 1990; Thomson, 1997). Proponents of global warming warn that increasing temperatures will increase the frequency of heat waves, increase

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This dissertation follows the style and format of *Bioresource Technology.*

frequency of severe weather events, disrupt plant and animal ecology, acidify the oceans, and increase ocean levels thereby decreasing available land (Houghton, 2005; Jacobson, 2008; Vitousek, 1994; Wentz et al., 2007). Assuming all energy inputs are from a renewable source, biofuel is carbon neutral (does not contribute to global warming). The carbon dioxide released during combustion is captured by plants that are then harvested and converted into biofuels; thus, there is no net increase in atmospheric carbon dioxide.

Although there is growing acceptance of global warming, there is much debate with numerous critics and experts making claims for and against it. If global warming *is* a reality, then biofuels are a carbon-neutral energy solution. If global warming *is not* real, the need for energy security is sufficient motivation for the United States to aggressively develop bioenergy.

1.2.2. Energy security

Energy security is a multi-dimensional concept characterized by conditions and policies that protect the US economy from short- and long-term increases in energy costs and decreases in supply (Parry and Darmstadter, 2003). Figure 1-1 shows the fraction of US petroleum consumption from net imports since 1950. The trend line in Figure 1-1 shows that US dependence on foreign petroleum has grown 1.29 percentage points per year since 1982. If this trend continues, the United States will be 100% dependent on foreign oil by 2038.

Because the United States overwhelming depends on foreign petroleum, it creates political, physical, and economic vulnerabilities. The long-term supply of oil from many OPEC nations (e.g., Libya, Saudi Arabia, Venezuela, Nigeria) as well as non-OPEC nations (e.g., Russia) is uncertain because of the geopolitical risk that these nations maybe overtaken by extremist governments willing to forfeit oil revenues to damage the US economy (Laney, 2006; Parry and Darmstadter, 2003; USDOE, 2009).

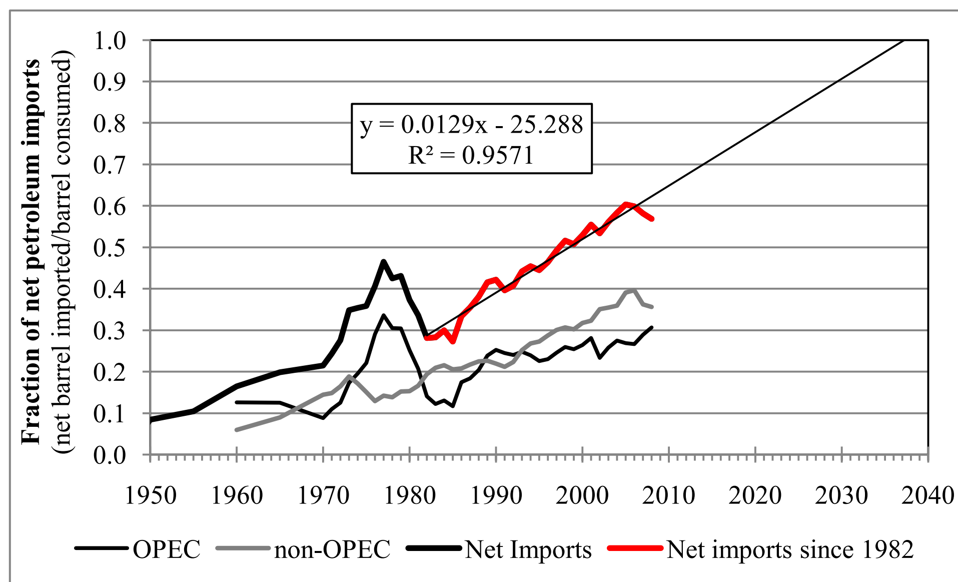


Figure 1-1. Fraction of US petroleum consumption from imports (2008 Annual Energy Review Table 5.1 & Figure 5.7 (EIA, 2009)).

According to Parry and Darmstadter (2003), the only way to reduce US vulnerability to world oil market volatility is to reduce the petroleum intensity of the gross domestic product (GDP). This may be accomplished by (1) increasing energy efficiency so that less oil is required for the same economic growth and/or (2) replacing petroleum with domestically derived biofuel.

The production strategy of OPEC nations is to maximize long-term revenue and market share by moderating oil prices so that non-OPEC nations are discouraged from developing higher-cost oil sources (Parry and Darmstadter, 2003). This strategy can increase the vulnerability of United States by reducing the economic incentive to develop biomass-based alternatives, as was the case in the late 1980's and 1990's. However, to be prepared for future energy crisis and oil market volatility, research and development of domestically derived petroleum alternatives (e.g., biofuels) must be aggressively pursued with a long-term focus.

Domestically derived biofuel production has a great capacity to stimulate the

local, state, and national economy. Biofuel will supplement domestic petroleum production and thus increase GDP. When Americans purchase imported petroleum, a large portion of the cost pays foreign producers and leaves the US economy. In contrast, virtually all the money spent on purchasing of domestically derived biofuel will stay in the United States. The feedstocks, infrastructure, and materials will be domestically resourced by US farmers and business. Commercial biofuel production will create jobs for construction workers, farmers, factory employees, business persons, engineers, and other peripheral personnel.

1.3. Practical constraints on any biorefinery

A biorefinery is analogous to an oil refinery; biomass, a raw complex material, is processed biologically, chemically, thermochemically, or a combination of the three to produce chemicals and fuel. There are numerous technologies each utilizing different feedstocks, chemical pathways, and producing different chemicals and/or fuels {Demirbas, 2007 #24;Fernando, 2006 #825;Huber, 2006 #140;Smith, 2010a #772}. When evaluating a biorefinery technology, regardless of the platform (i.e., biological, chemical, thermochemical), there are practical constraints that must be navigated:

- Uses waste and/or lignocellulose as feedstock
- Is economical at modest economy of scale
- Produces hydrocarbon fuel
- Has competitive retail price
- Is truly sustainable

Those technologies most able to negotiate these challenges will be the most likely to (1) significantly impact energy supply and (2) be economically viable without government subsidy.

1.3.1. Uses waste and/or lignocellulose as feedstock

To replace imported petroleum (or even a significant fraction) with domestically produced biofuel, a large biomass resource is needed. There are four categories of crops considered as feedstock: lignocellulose, sugar, starch, and oil. Of these, lignocellulose is

the most abundant, the most productive (i.e., annual mass per land area), and least expensive. In 2008, the United States consumed ~100 quadrillion Btu (quads) of fossil fuel energy of which 37 quads came from petroleum. Annually, about 2700 quads of biomass are produced globally by photosynthesis (Chen et al., 2003; EIA, 2007), so there are sufficient quantities of lignocellulosic biomass if it can be collected. Compared to corn grain (~5 dry ton/(acre·yr)), energy crops (e.g., forage sorghum, 15–20 dry ton/(acre·yr)) can produce 3–4 times more biomass per acre, thereby dramatically reducing land requirement. On an energy basis, lignocellulosic biomass is less expensive that petroleum ($5–20 USD per barrel of oil equivalent versus $50–140 USD) (Huber et al., 2006). Therefore, it is realistic that biomass can displace a significant fraction (or all) of US petroleum needs.

Because of their low or negative value, the organic fraction of municipal solid waste (MSW) (i.e., trash) and agricultural residues (i.e., plant material remaining after harvest) are economically attractive feedstocks for near-term commercialization. Although the supply of MSW and agricultural residues is insufficient to completely displace imported petroleum, its low cost improves process economics for initial manufacturing plants, which are more expensive and less optimized than subsequent facilities. Because MSW and agriculture residues are predominately lignocellulose, theoretically, the same technology used to convert lignocellulosic crops into chemicals and fuels could be applied.

Because of its abundance, productivity, and cost, lignocellulose and waste are the only resources able to providing enough feedstock to replace petroleum. Lignocellulose feedstocks are characteristically recalcitrant (i.e., difficult to degrade), typically have a high moisture content, and are not sterile. A biorefinery must be able to overcome these technical challenges. Recalcitrance is primarily a function of biomass crystallinity and lignin content; thus, effective pretreatment may be required (Chang and Holtzapple, 2000; Chang et al., 2001; Sun and Cheng, 2002). The high moisture content influences transportation and process steps, especially for thermochemical platforms (Phillips et al., 2007). For biological process, especially those that use a monoculture, contamination

from microorganisms on the feedstock could ruin product and/or require added capital and operating cost to prevent contamination (Smith et al., 2010). Additionally, to maintain a constant feedstock supply, large-scale biorefineries will likely use different lignocellulose resources depending on the season; thus, a biorefinery must be able to process a variety of feedstocks.

1.3.2. Is economical at modest economy of scale

Collection and transportation of biomass is a primary logistical issue for any biorefinery (Mahmudi and Flynn, 2006). Biomass is dispersed over large farms, in varying proximity to each other and the biorefinery. Because biomass typically has high moisture content and a low energy density relative to fossil fuels, transportation can contribute a significant cost. Biomass transportation over 100 km is generally considered cost prohibitive (Kumar and Sokhansanj, 2007; Mahmudi and Flynn, 2006; Searcy et al., 2007). In other words, the amount of biomass that can be collected in a 60-mile radius dictates the size of the biorefinery.

A 2005 National Renewable Energy Lab (NREL) study estimated the biomass resource availability in the United States. The evaluation included agricultural and forest residues, and production of switchgrass and poplar wood on Conservation Reserve Program (CRP) lands (Figure 1-2).

Biorefineries that require large economies of scale (>3,000,000 dry tonne/yr) are limited to locations that can provide a large supply of feedstock. If economical locations are too limited, then US biofuel capacity is limited to the biomass that can be collected in those key locations. Conversely, a biorefinery that is economical on a smaller scale (<2,000,000 dry tonne/yr) is viable in more locations; thus, more biomass can be utilized and US biofuel capacity increases. To illustrate this point, Figure 1-2 shows the biomass density (dry tonne/($km^2 \cdot yr$)) for each county. Assuming a 100-km collection radius, a 750,000 dry tonne/yr biorefinery (~5 million gal biofuel/yr) would need the average biomass density to exceed 24 dry tonne/($km^2 \cdot yr$) (i.e., majority of area in a 100-km radius circle on Figure 1-2 must contain colored counties). A 7,500,00 dry tonne/yr

biorefinery (~50 million gal biofuel/yr) would need an average biomass density of 238 dry tonne/($km^2 \cdot yr$) (i.e., the area in a 100-km radius circle on Figure 1-2 must be purple and red counties).

1.3.3. Produces hydrocarbon fuel

To utilize current automobiles and existing infrastructure (i.e., pipelines and fueling stations), biorefineries must be able to produce hydrocarbon fuels (i.e., gasoline, diesel, jet fuel) (Rowlands et al., 2008). A biofuel other than hydrocarbons, will likely require consumers to purchase new vehicles and manufactures to retool and/or replace the current petroleum infrastructure, which is valued in trillions of dollars (Mader, 2006). Biomass-derived hydrocarbon fuel is compatible with existing infrastructure; thus, it can be *immediately* distributed and used by the public. Significant modification to the current infrastructure will add cost and delay transition from foreign oil to domestically produced biofuel. Assuming the fuel has the same properties and specifications as current hydrocarbon fuels, consumer acceptance is virtually guaranteed. In contrast, a fuel with different properties that requires a different vehicle may take a long time for consumers to embrace.

1.3.4. Has competitive retail price

Bottom-line economics will ultimately dictate the viability of any technology. To attract consumers to purchase biofuel, the cost needs to be less than or equal to the present-day cost of petroleum. Without price-competitive biofuel, public acceptance will be limited to the small population segment willing to pay more for environmentally conscious fuel.

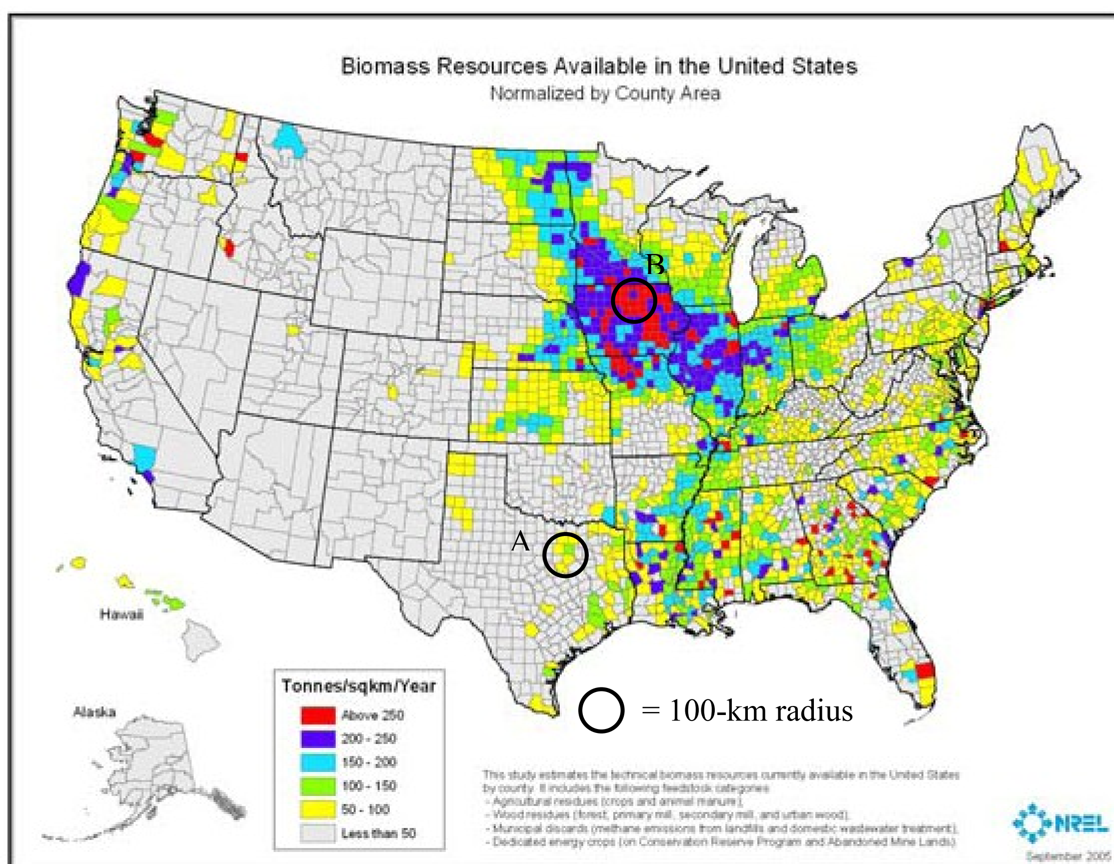


Figure 1-2. Biomass resources available in the United States normalized by county area (dry tonne/(km^2·yr)) (Milbrandt, 2005). Circles A and B represent example 100-km biomass collection radius areas capable of supplying 750,000 and 7,500,000 dry tonne/year biorefineries, respectively.

1.3.5. Is truly sustainable

A parallel motivation to develop biorefineries is to increase sustainability and decrease impact on the environment. However, the sustainability of a biorefinery is complex and difficult to define. The following aspects of the biorefinery life cycle must be considered: land use, crop selection, soil health, water use (agricultural and process), waste/emissions, and carbon-neutrality (Laney, 2006). If a biorefinery is not sustainable, the environment could be damaged, natural resources could be unnecessarily depleted,

and the human health, well-being, and quality-of-life could be compromised. The following subsections briefly discuss the major sustainability concerns. The questions and examples posed are intended to provoke thought on the issue rather than imply impossibilities. Many of these concerns *can* be addressed in a sustainable way; others are topics of on-going research. Development of a truly sustainable biorefinery is a challenging and intricate task that will require multi-disciplinary effort, and corporate and public commitment.

Land use

To produce biomass for a mature biomass-based economy, large amounts of farmland will be required. Assuming no loss of food-designated farmland, where will the new farmland be located? Can marginal lands be used? Will disturbing the land to create a biomass farm decrease biodiversity, disrupt ecology, and/or increase deforestation? Will humanity's future demand for energy compromise preservation of national parks, state parks, and other special lands?

Crop selection

The choice of energy crop will influence the effect on local ecology, water usage, farming practices, pesticide usage, and likelihood of plant disease. For example, a genetically engineered plant and/or invasive plant species could potentially get out of control and damage local ecology by decreasing plant diversity. Decreased plant diversity influences animal diversity and increases opportunity for plant disease and pests, which is a common problem with current agriculture. However, the right choice of plant(s) could increase diversity by providing an additional plant that a farmer may put in rotation.

Soil health

Closely related to crop selection is concern for soil health. For a given crop and corresponding farming practices, what are the impacts on soil erosion, and the carbon and nutrient content of the soil? How much fertilizer is required? Does the crop help sequester carbon in the soil because of its root structure?

Water use (agricultural and process)

The availability of fresh water is a growing concern (Alley et al., 1999; Gleick, 1998). To be sustainable, biorefineries need to be net-zero water consumers (or net producers). Crops and processes that are water intensive could potentially strain the municipal water supply and increase water cost. Depending on the process, a biorefinery could be a net producer of fresh water. For example, if sewage sludge, which is mostly water, was used as the nutrient supply, it is conceivable that a fermentation process could decontaminate the feed. Further, distillation during dewatering can purify the water that was in the feedstock.

Waste/emissions

Inevitably, any biorefinery will have waste. To reduce political, environmental, and regulatory challenges, waste disposal issues and/or hazardous byproducts need to non-existent or effectively mitigated in an environmentally conscious way. The purpose of a biorefinery is to produce chemicals and fuels *in an environmentally friendly fashion*; thus, if a biorefinery produces environmental hazards (or perceived hazards), there could be political backlash against the process. For example, wastes from biological processes can have a stigma because they contain microorganisms and may raise concerns for public health and potential ground water contamination. Depending on the circumstance, this may be an actual hazard or a just a perceived hazard, nonetheless, it will cause political strife and may unnecessarily increase waste disposal costs. Not-in-my-backyard (NIMBY) issues must be mitigated to gain public acceptance. For example, if a biorefinery produces foul odors, local citizens may protest the facility location.

Carbon-neutrality

Theoretically, biofuel production is carbon neutral. The facilities could be powered by renewable electricity (e.g., solar, wind, hydropower) or electricity generated on-site from combustion of waste residues. Farm equipment (e.g., tractors, harvesters, etc.) could run on the biofuel; thus, requiring no fossil fuel. This scenario is the vision

for future biorefineries; however, economics could preclude carbon-neutrality.

Because biomass has significant oxygen content, hydrogen is need to upgrade products into hydrocarbon fuels. Currently, steam reforming of natural gas (fossil fuel), which produces carbon dioxide, is the least expensive way to produce hydrogen. Biorefineries can produce hydrogen from biomass or waste residues, but require additional capital and/or loss of carbon as carbon dioxide (i.e., less biomass carbon is converted to fuel). The most economical way for a biorefinery to upgrade products may be to use fossil-fuel derived hydrogen. Likewise, the purchase of coal-derived electricity might be more economical than renewable alternatives.

1.4. The MixAlco™ process

The MixAlco™ process (Figure 1-3) is a "biorefinery" that converts any biodegradable biomass into useful chemicals and fuel (Holtzapple and Granda, 2009; Holtzapple et al., 1999; Smith et al., 2010). Although some substrates (e.g., food scraps and office paper) are easily digested, most lignocellulosic biomass must be pretreated with lime and oxygen/air to increase digestibility. The biomass is then fermented by a mixed culture of acidogens to produce two- to seven-carbon carboxylic acids, which are buffered with calcium carbonate or ammonium bicarbonate. The fermentation broth is clarified, concentrated, and dried to produce carboxylate salts, a "biocrude" that can be chemically converted to chemicals and fuels.

Mixed-acid fermentation is a key step in the MixAlco™ process (Figure 1-3) because it dominates the capital costs, and determines the overall rates and yields. Mixed-culture acid fermentation is ideal for a biorefinery for the following reasons: (1) no enzyme addition, (2) no genetically modified microorganisms or mono-cultures, (3) no contaminates, (4) adapable to feedstock fluctuations, and (5) low capital and operating costs. The mixed-culture acid fermentation employs similar microorganisms as biomethane fermentations, except methanogens are inhibited with iodoform (Ross, 1998). Granda et al. (2009) showed that the MixAlco process can produce gasoline for less than \$3/gal; thus, the MixAlco process is an attractive source of renewable energy.

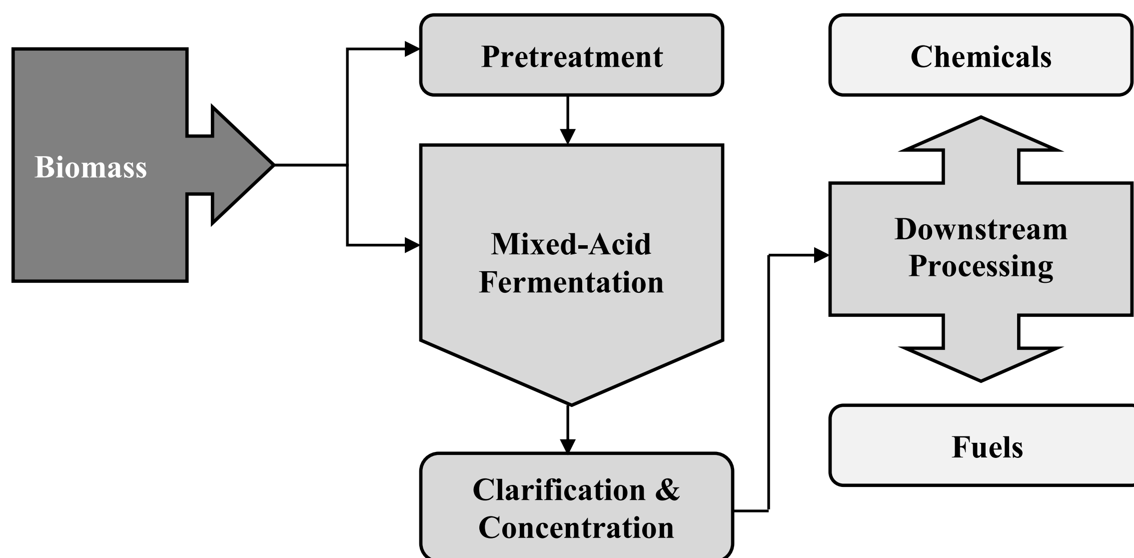


Figure 1-3. MixAlco process block flow diagram.

Typically, four fermentors (Figure 1-4) are used to create a countercurrent fermentation "train" (Agbogbo, 2005; Aiello-Mazzarri, 2002; Domke et al., 2004; Fu, 2007; Ross, 1998). The first fermentor is fed with the most reactive (fresh) biomass, but has the highest product carboxylic acid concentration (greatest product inhibition). The last fermentor has the most recalcitrant (digested) biomass, but has the lowest product concentration (least product inhibition). This countercurrent strategy achieves both high product concentration and high conversion.

The main objectives of this research were to (1) operate a 4000-gallon (pilot) countercurrent submerged fermentation at steady state (Sections 2 & 3), (2) compare steady-state performance of pilot fermentation with continuum particle distribution model (CPDM) predictions (Section 4), (3) investigate the behavior of nitrogen and different nutrient feeding strategies on fermentation performance (Section 6 & 7), and (4) investigate influence of carbohydrate-nutrient blend and carbon-nitrogen ratio on

fermentation performance (Section 8). A spin-off development from this research was the Slope Method (Section 5) for analyzing semi-continuous steady-state data, which dramatically reduces error of average material rates calculated from semi-continuous data.

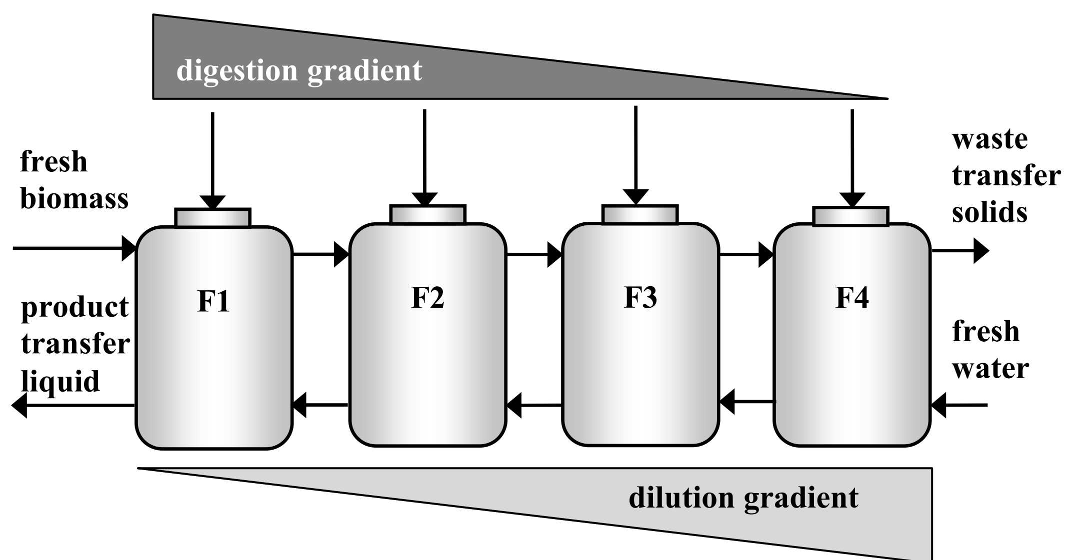


Figure 1-4. Four-stage countercurrent fermentation train with digestion and dilution gradients.

2. SUMMARY OF PILOT FERMENTATION OPERATIONS

The pilot plant (Figure 2-1) operations discussed in this dissertation (Sections 2–4) occurred between December 2005 and October 2008. The key objective was to achieve steady state so that performance (i.e., acid concentration, yield, conversion) could be compared with CPDM predictions, which are presented in Section 4. While working to satisfy this objective, much was learned about large-scale fermentation operation. The purpose of this section is to provide a timeline of events (Section Pilot plant operations), discuss material handling and equipment issues (Section Solids handling and equipment issues), tools (Section Tools), and the stability of product liquor in outdoor storage (Section Preservation of product liquor).

Figure 2-1. Picture of front side of pilot plant (Summer 2006).

2.1. Pilot plant operations

2.1.1. Renovation (December 2005–September 2006)

Upon assuming management of the MixAlco pilot plant (December 2005), the state of the facility was disorganized, cluttered, overly dirty (i.e., biomass on all

equipment), and dysfunctional. There were many unnecessary safety hazards that were the result of insufficient management, planning, and design. With regard to fermentation operation, insufficient infrastructure and tools existed to execute tasks and make measurements necessary to achieve steady state.

For the first month, operations continued as had been established previously (Moody, 2006). During this time, the pilot plant was thoroughly evaluated to determine organizational, maintenance, and infrastructure needs. Safety hazards were identified. Task lists were created and priorities established.

Beginning in January 2006, to address safety, organizational, and infrastructure issues, operations were suspended. Safety, functionality, access, and maintenance were considered in all renovation designs. Over the following nine months, the pilot plant was renovated. The following subsections highlight key improvements.

Safety

Safety is a top priority in any workplace. Unfortunately, the state of the pilot plant in December 2005 included many safety hazards. The original construction and installation of equipment routed many conduits and pipes across both major walkways creating obstacles and tripping hazards. Many pieces of equipment and infrastructure had protruding objects that posed tripping and/or snagging hazards. The uncleanliness, clutter, and disorganization also created unnecessary hazards. To address safety issues, the following actions were taken:

- Piping that required operators to step over or duck under to pass, were either removed or rerouted (Figure 2-2).
- A safety shower and eye wash station were installed (Figure 2-3).
- Tripping and/or snagging hazards that could not be removed were painted with yellow caution paint (Figure 2-3).
- Walkways were outlined with yellow stripes.
- Containers of unused chemicals were given to the university's chemical waste disposal.

- Operators were required to take safety training.
- Operators were required to wear hardhats, safety glasses, long pants, and closed toe shoes.

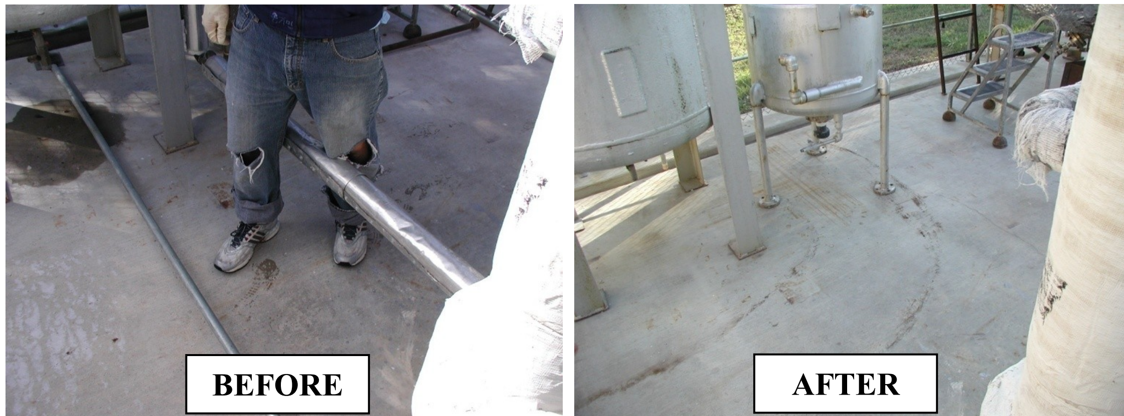


Figure 2-2. Before and after pictures of conduit and pipe that were removed from walkway.

Figure 2-3. Pictures of places where safety yellow paint was used identify potential safety hazards. Safety shower and eye wash station are shown in right-most picture.

Cleanliness and organization

Operation of the pilot plant fermentation is an inherently dirty task because biomass is spilled when being added, removed, or transferred between fermentors (Figure 2-4). The original design made regular cleaning (i.e., spraying biomass off equipment and concrete slab) difficult because most equipment was low to the ground. As a result, regular cleaning was over looked and spilled biomass accumulated. The spilled biomass created slipping hazards, was unsightly, and attracted insects. Additionally, organization and storage of items had been neglected; the limited workspace was unnecessarily constrained by unused equipment, materials, and supplies.

An unclean and disorganized workspace exacerbates safety concerns, employee morale, productivity, and public appeal; thus, by improving the cleanliness and organization, other areas of concern were simultaneously improved. To improve cleanliness and organization, the following actions were taken:

- Tools, materials, supplies and equipment were cleaned, sorted, and stored.
- Damaged and/or unnecessary items were discarded (trash) or taken to TAMU surplus.
- To store large items, an area in the adjacent parking lot was reserved.
- To store expensive, small, and/or weather-sensitive items, a storage room inside the University Services Building (USB) (adjacent building) was organized and shelves installed.
- To maintain cleanliness, a pressure washer was purchased and the pilot plant was pressure washed after each transfer.
- To create functional workspace, equipment was reorganized and/or removed.
- To provide counter-top workspace, a work bench was built in the tool shed.
- If possible, equipment and infrastructure was elevated so the concrete slab could be more easily pressure washed.
- Squeegees were purchased to recover spilled biomass and aid cleaning.

Figure 2-4. Biomass spilled during a transfer (June 2007).

Functionality

Functionality is critical for effective and efficient use of equipment and workspace. The original routing of pipes and conduits made moving and accessing equipment and utilities difficult and unsafe. In some instances, piping was placed in such a way that pilot plant functionality was negated (e.g., steam line in drain canal, Figure 2-5). The poor design and construction of the fermentor recycle loops directly interfered with fermentor access and made the use of recycle loop ports and valves unnecessarily difficult. The top recycle loop ports (i.e., 2-inch ball valve with male quick connect) protruded over the top fermentor port holes. The bottom ports were angled down so that connecting a transfer hose was unnecessarily difficult (see figure on page 21). The redirection valve was located on the top of the fermentor, which was inconvenient to operators working on the ground.

Previous pilot plant work tested a percolation/filtration system for handling fermentation material. Because the design proved ineffective (Moody, 2006), it was no longer used; however, the piping inside the fermentor was not removed. This hindered mixing and reduced the effective fermentation volume (Figure 2-6). Additionally, other unused equipment cluttered the pilot plant thereby reducing workspace. To make the pilot plant workspace more functional, the following improvements were made:

- The water line, which was unsecured piping lying on concrete slab, was relocated to elevated (36" above slab) brackets along the fence (Figure 2-3 and Figure 2-5).
- To open the drain canal, the lower steam line, which ran in the drain canal, was removed; clogs in the drain were removed and drain canal was flushed (Figure 2-5).
- The percolation system inside the fermentors was removed because it did not work and hindered mixing (Figure 2-6).
- Fermentor recycle loops were redesigned to be more ergonomic and not obstruct access to the fermentor port hole (Figure 2-7).
- To increase mobility, wheels were added to small equipment.
- To make the sludge pump switch box more accessible, it was relocated from the back fence, which was an out-of-the-way location, to between Fermentors 1 and 2 where operators commonly work.

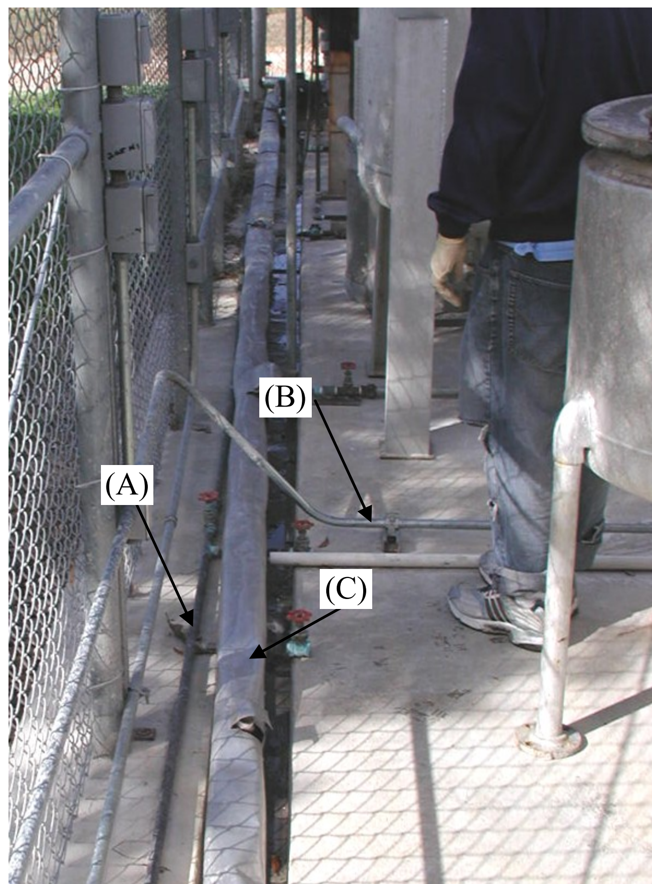


Figure 2-5. Picture of obstructions around drain canal before renovation: (A) water line, (B) electrical conduit, and (C) lower steam line.

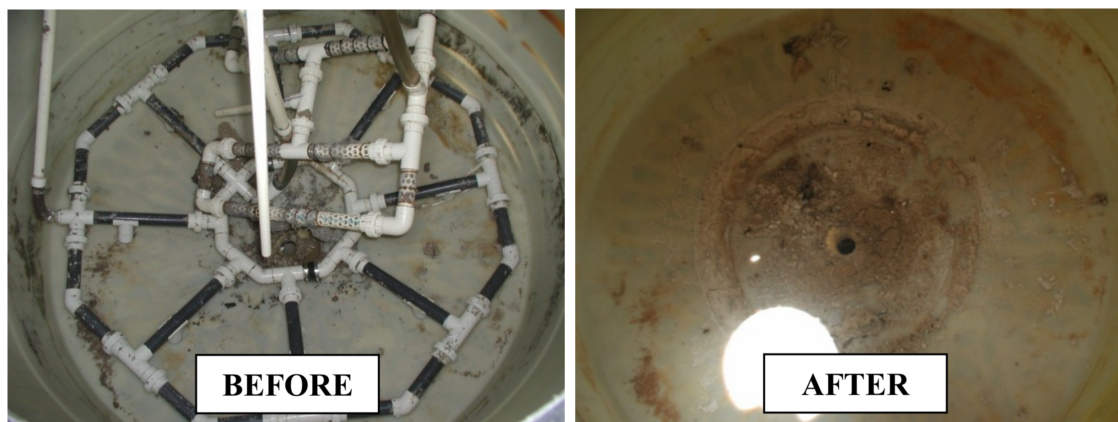


Figure 2-6. Pictures inside fermentors before and after percolation system was removed.

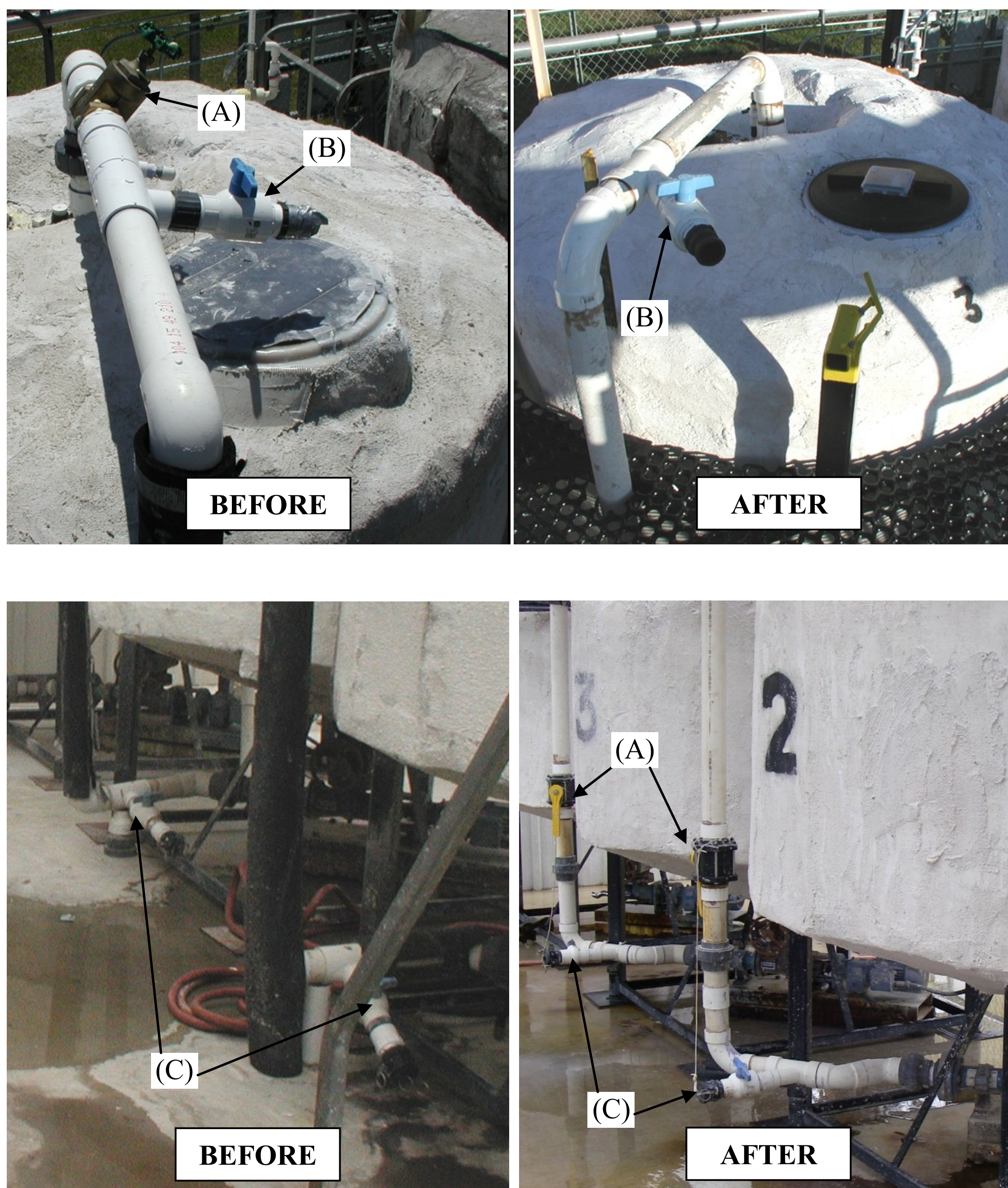


Figure 2-7. Pictures before and after recycle loop was redesigned with components identified: (A) redirection valves, (B) top recycle loop port, and (C) bottom recycle loop port.

Fermentor and utility access

When renovating the pilot plant, the greatest need was improved fermentor access. Previously, rolling ladders were used to access the fermentor port hole to mix and/or add substrate (Figure 2-8). The rolling ladders were unstable (safety hazard) and provided an insufficient vantage point to interface with the fermentor port hole. Additionally, the rolling ladders interfered with ability of operators to move and use transfer hoses on the ground. To improve fermentor access, a catwalk was installed on the port-hole side of the fermentors (Figure 2-8).

In addition to fermentor access, there was a need to improve access to utilities (i.e., water and electric) and the closed-loop water heating equipment that maintained fermentor temperature. The previous equipment layout was unsafe to navigate around and made maintenance very difficult (Figure 2-9). Much of the plumbing was associated with the abandoned percolation/filtration system. Most of the equipment was placed on the ground and blocked access to the drain canal; thus, pressure washing around this equipment was almost impossible. To install the catwalk, all equipment was removed. After the catwalk was installed, elevated shelves were added to the catwalk vertical supports. The water heaters and pumps were reinstalled on these elevated platforms (Figure 2-9). The unused equipment and materials were placed in storage.

Maintenance

To maximize usable life and performance, equipment maintenance is essential. To maintain equipment and infrastructure, the following improvements were made:

- Insulation was repaired on fermentors and stucco was applied to seal holes and gaps (Figure 2-10).
- Equipment maintenance (e.g., pump lubrication, oil change) was performed and a maintenance schedule was created.
- To counteract weather, rust was removed from metal work and rust-resistant paint applied.

Figure 2-8. Pictures of fermentor access before and after catwalk construction.

Figure 2-9. Pictures of water heaters and plumbing behind fermentors before and after renovation.

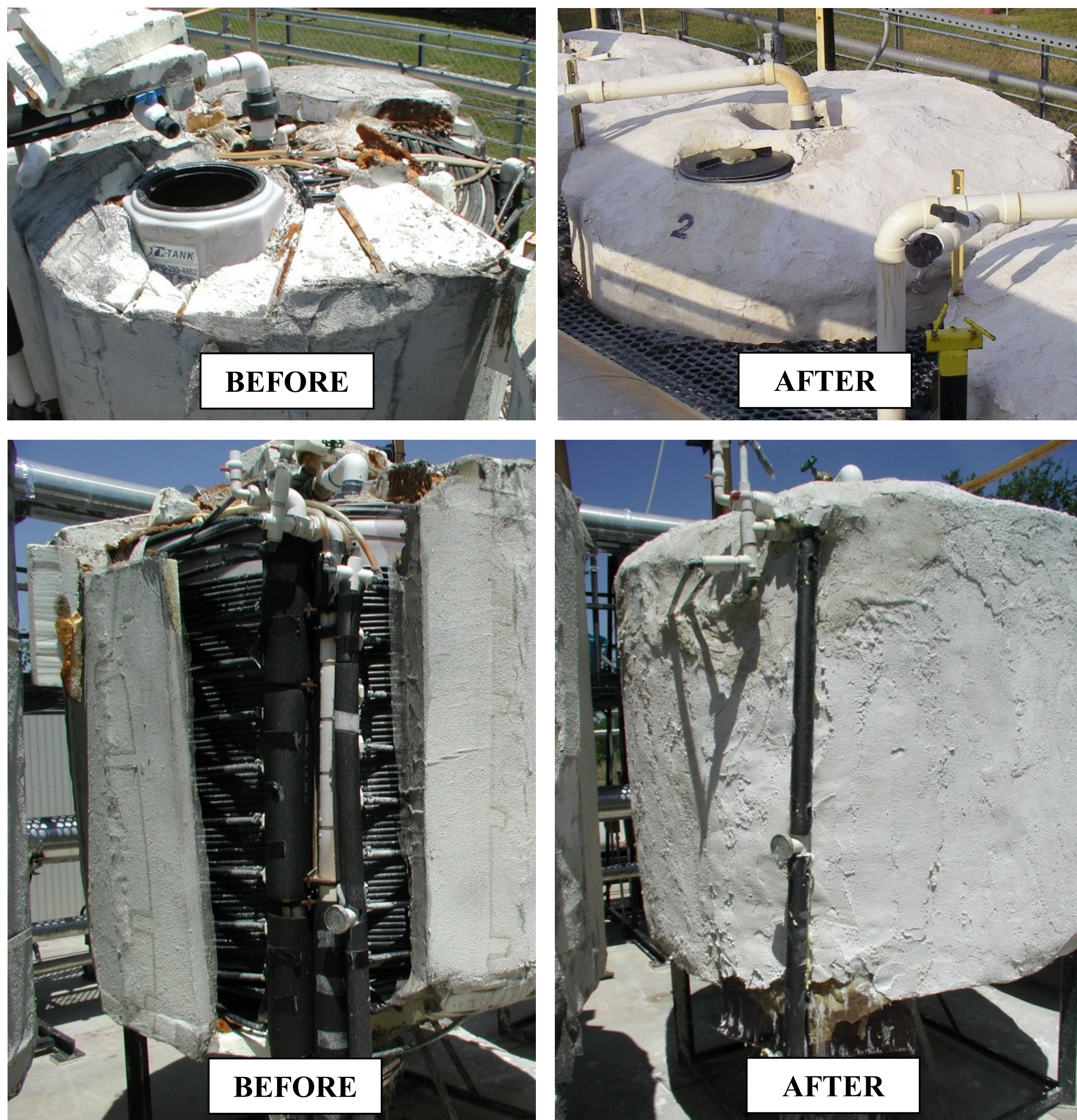


Figure 2-10. Pictures before and after fermentor insulation was repaired.

2.1.2. Fermentation components and start-up (October 2006)

By the end of September 2006, all critical renovations were complete and the fermentators were ready to be refilled and operated. Because the focus of the pilot plant fermentation was steady-state operation (Section 4), shredded office paper and wet

chicken manure were selected as feedstock because they do not require pretreatment. The following outlines each fermentation component and the start-up process.

Inoculum

To collect marine inoculum, a trip was made to Galveston, TX on September 23, 2006. To obtain sand that was rich in anaerobes, a hole was dug in the beach to a depth of 2–3 feet (Figure 2-11A). It was desired to collect sand that contained organic matter, which typically made the sand black, and had a foul odor indicating microbial activity. To remove large particles that could damage sludge pumps, screens were placed on top of the 5-gallon collection buckets through which the sand was sieved (Figure 2-11B). Two trucks and five persons collected 40 5-gallon buckets full of beach sand. Upon returning Texas A&M University, inoculum was placed in a refrigerated room (40˚F) until use.

Figure 2-11. (A) Picture of student workers collecting and screening sand from Galveston, TX beach. (B) Bucket-top screens used to eliminate large particles (e.g., shells, debris) that could damage pumps.

Office paper

Previous pilot fermentations (Moody, 2006) used large bails of coarsely shredded office paper from a document destruction company. This paper supply contained many objects (Figure 2-12) that could damage the progressive-cavity pumps. The previous protocol only the largest most obvious objects; small objects (e.g., metal paper clip) were overlooked and damaged pumps because they were easily funneled into the pump intake and damaged working parts.

To enhance detection of metal objects, a handheld metal detector was purchased. To remove all hazardous objects, a paper sorting table was constructed using no metal screws or nails, which prevented interference with the metal detector. Initially, paper was sorted using student labor. Because the paper sorting process was very tedious, labor cost greatly inflated the effective cost of the office paper (~10 lb sorted paper/(hr·person) @ \$7/hr = ~\$2,800/sorted ton); thus, paper sorting was stopped.

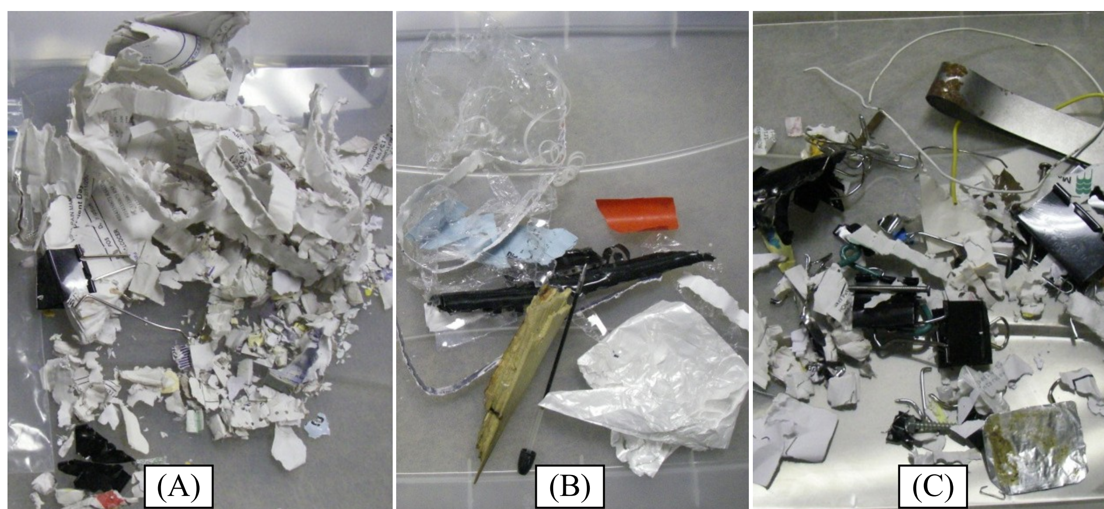


Figure 2-12. Pictures of (A) representative sample of paper with objects, (B) non-metalic objects, and (C) metalic objects found in paper supplied by document destruction company.

To obtain object-free paper without objects that could damage pumps, shredded paper was purchased from the Texas A&M University Recycling Center. Although this paper supply was much "cleaner" than the previous source, operators had to screen paper for plastic bags and whole documents prior to adding to fermentor. Plastic bags would tangle around pump rotor. Whole documents, whole sheets of paper, and long strips of paper could hinder mixing and clog the pump. For this reason, confetti-, cross-, and fine-cut shredded paper was preferred to strip-cut shredded paper. The properties of office paper and chicken manure are shown in Table 2-1.

Chicken manure

To have enough manure for start-up, a trailer equipped with two feeding troughs (69" × 23" × 23") were used to collect chicken manure. Five-gallon buckets were used for subsequent collection and on-site storage of chicken manure. Chicken manure was provided by Feather Crest Farms (14374 E. Hwy 21, Bryan, TX), which produces eggs. Manure was always used as-is (i.e., not dried).

Table 2-1. Feedstock properties.

	Office paper	**Fresh chicken manure**
Moisture content, *M* (g H_2O/g wet sample)	0.067 ± 0.02	0.691 ± 0.08
Ash content, *I* (g ash/g dry sample)	0.136 ± 0.02	0.514 ± 0.05
Carbon content, C (g C/100 g wet sample)	36.3 ± 0.7	10.2 ± 0.7
Nitrogen content, N (g N/100 g wet sample)	0.27 ± 0.08	1.45 ± 0.2
Carbon-nitrogen ratio (g C_{NA}/g N)	135.3 ± 40	7.0 ± 1.2

Error values represent one standard deviation

pH buffer

Fifty-pound bags of calcium carbonate were purchased from Producers Cooperative Association (1800 N. Texas Ave., Bryan, TX; $5.20/bag). Initially, buffer

was added according to Table 2-2. Subsequent addition of buffer was unnecessary because the fermentation self-regulated between pH 5.5 and 7.0 (typically pH 6.1).

Methane inhibitor

To inhibit growth of methanogens, 200 mL of 40 g/L iodoform-in-200-proof-ethanol solution was added to each fermentor daily prior to mixing. If, upon compositional analysis of gas headspace, methane production was detected, double dosages would be added until methanogeneisis was suppressed.

Media

For laboratory fermentations, deionized water is deoxygenated by boiling and addiing reducing agents. Deoxygenation was not practical for pilot fermentations. To reduce the anti-microbial effect of halogenated water (chlorine), city water was passed through a granular activated carbon (GAC) bed before use.

Fermentor loading

Because fresh office paper is very fibrous and over-addition can make fermentor contents too thick to mix and pump, fermentors were loaded in three increments. This strategy allowed time for the macro structure of the paper to breakdown so that mixing and pumping could be maintained. Table 2-2 outlines components and amounts fed to each fermentor during Loadings 1–3.

When adding fresh paper, wetting and mixing are critical to reduce clumping so that mixing and pumping is maintained. For the first loading, before adding substrate, each fermentor was filled with 300 gallons of water. The majority of paper (50–60%) was added, mixed, and wetted with water in the tank. Then, the remaining paper, chicken manure, calcium carbonate, and inoculum were gradually added. As needed, water was sprayed in the fermentor to help wet paper and incorporate components. Water was added to reach a final volume of 850 gallons. Between loadings, batch operation was maintained. Before Loadings 2 and 3, liquid was removed to accommodate the volume of fresh material.

To determine the corresponding influence on performance, the amount of inoculum added to each fermentor was varied (Figure 2-13). Figure 2-13 shows that the inoculum loading had no discernable influence on acid concentration. At the end of fed-batch operation, F1, F2, and F4 had similar acid concentrations (15–16 g/L), which were loaded with 15, 25, and 50 gal of Galveston beach sand and water (inoculum). Much of the noise can be attributed to insufficent mixing because the fresh biomass was very thick.

Table 2-2. List and amounts of components added to each fermentor for each loading during pilot fermentation start-up. All weights are on an as-is basis. *Water was added until the desired final volume was obtained.

	Component	Units	F1	F2	F3	F4
1st loading (Sept. 27, 2006)	office paper	lb	400	400	400	400
	wet chicken manure	lb	335	335	335	335
	inoculum	gal	5	25	35	50
	methane inhibitor	g	32	32	32	32
	buffer	lb	180	180	180	180
	water (final volume)*	gal	850	850	850	850
2nd Loading (Oct. 20, 2006)	liquid removed	gal	50	65	50	75
	office paper	lb	200	198	200	204
	wet chicken manure	lb	208	200	199	206
	inoculum	gal	---	---	---	---
	methane inhibitor	g	32	32	32	32
	buffer	lb	100	100	100	100
	water (final volume)*	gal	850	850	850	850
3rd Loading (Oct. 27, 2006)	liquid removed	gal	65	65	50	65
	office paper	lb	202	304	305	304
	wet chicken manure	lb	251	247	249	249
	inoculum	gal	---	---	---	---
	methane inhibitor	g	32	32	32	32
	buffer	lb	---	---	---	---
	water (final volume)*	gal	850	850	850	850

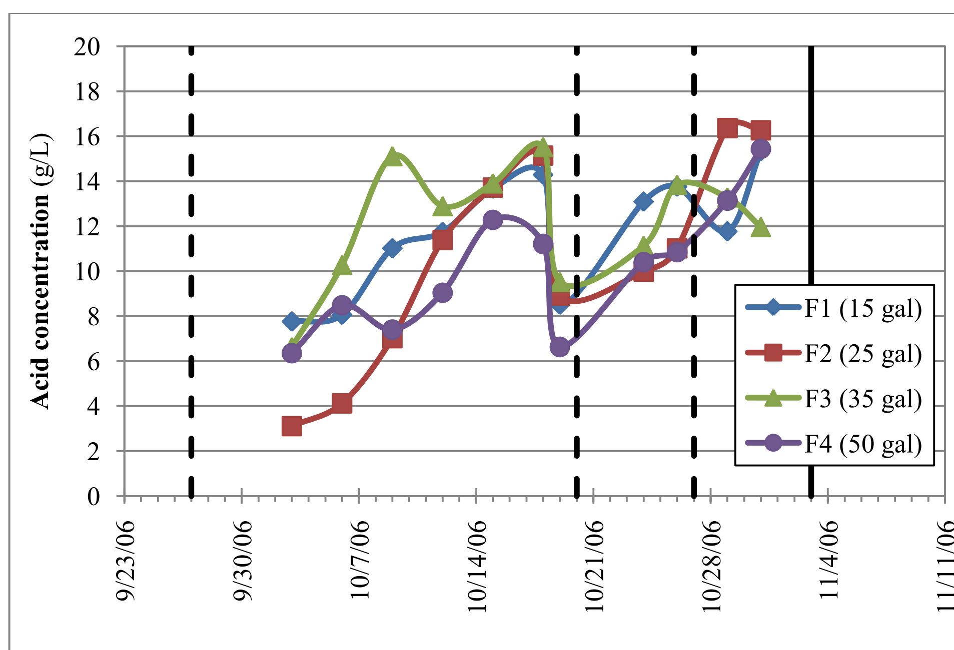


Figure 2-13. Acid concentration for Fermentors 1–4 while in fed-batch operation. Fermentors 1–4 were loaded with 15, 25, 35, and 50 gallons of Galveston, TX beach sand and water, respectively. The dashed lines indicate the incremental loadings. The solid black line indicates the beginning of Trial 1 (i.e., countercurrent operation).

2.1.3. Trial 1 (November 2006)

Because the pilot plant was not automated, countercurrent movement of transfer solids and transfer liquids was accomplished by discrete transfer of material on a fixed frequency (typically twice per week). Previous attempts at steady-state operation failed (Moody, 2006) because the mass of solids and liquids transferred were assumed rather than based on measurements and a material balance.

To apply a material balance to the pilot fermentation, the volume and dry solid concentration must be measured for each fermentor. Prior to renovation, volume measurement made by visual estimates using 100-gallon-volume markings on the inside fermentor wall, which estimated the volume within 50 gallons. Installation of the catwalk and mixing ring provided a reference from which volume could be more accurately measured. (For more details see Section 2.3.) Although a technique existed to measure the dry solid concentration (Method 4-1A; Section 3.4.2), it was underdeveloped because previous pilot fermentation work did not apply a material balance; therefore, this measurement was not *perceived* as critical to steady-state operation.

Once the fermentors were filled, Trial 1 began with the first countercurrent transfer on November 3, 2006. The second transfer scheduled for November 6 was forgone because dry solid concentration data indicated the total solids in each fermentor had increased since the previous transfer; this observation contradicted expectations. Over the following three days, in preparation for the next transfer attempt (November 10), the dry solid concentration technique was evaluated and new techniques were proposed. Because validation of a dry solid concentration method is difficult and time consuming, transfer quantities were assumed (i.e., not calculated from mass balance) and executed. The next transfer on November 13 was dictated by a mass balance. On November 17, countercurrent operation was suspended to research dry solid concentration techniques that had greater precision and develop a computer-based mass balance calculator. The following summarizes the issues that contributed to the discontinuation of Trial 1:

- Mass balances were oversimplified and included too many assumptions (e.g., moisture content and density of transfer solids and liquid were guessed).
- Manual calculation of mass balances (i.e., calculated on paper with hand-held calculator) was time consuming, which held up transfer activities until calculations were complete.
- Measurement of dry solid concentration was logistically inconvenient (i.e., transport of samples and data sheets between pilot plant and laboratory) because there was no oven on site.
- Solid concentration data (Method 4-1A&B, Section 3) were inconsistent and trends were illogical; samples did not dry in required time.
- Turn-around time for solid concentration data was several days; thus, mass balances were not based on real-time data.

To address these issues, a spreadsheet-based mass balance calculator was created, an oven was obtained, and research was done to improve the precision and accuracy of dry solid concentration measurement (dry solid concentration Methods 4-1B&C, 4-2A, and 4-7A evaluated). To help vet different solid concentration techniques, periodic practice transfers were made, but countercurrent operation was not sustained. Detailed discussion of solid concentration methods is reserved for Section 3. Tables 2-3 and 2-4 summarize the controllable and normalized operating parameters, respectively, for Trial 1.

Table 2-3. Summary of controllable operating parameters for Trial 1. Error represents two standard deviations.

Trial 1			
Parameter	**Units**	**Set point**	**Actual**
Temperature, F1	˚C	40	43.3 ± 3.4
Temperature, F2	˚C	40	45.1 ± 6.6
Temperature, F3	˚C	40	43.3 ± 2.6
Temperature, F4	˚C	40	43.6 ± 4.6
Transfer frequency	transfers/week	2	1.5 ± 2
paper feed rate	dry lb/transfer	50	50 ± 4
Chicken manure feed rate	dry lb/transfer	13	13 ± 2
Product transfer liquid rate, L_1	gallons/ transfer	35	38 ± 20
Total volume, F1	gallons	850	840 ± 44
Total volume, F2	gallons	850	820 ± 36
Total volume, F3	gallons	850	818 ± 22
Total volume, F4	gallons	850	845 ± 36
Dry solids conc., F1	dry lb/ bulk gal	0.85	1.105 ± 0.27
Dry solids conc., F2	dry lb/ bulk gal	1.05	1.188 ± 0.34
Dry solids conc., F3	dry lb/ bulk gal	1.05	1.030 ± 0.15
Dry solids conc., F4	dry lb/ bulk gal	1.05	1.435 ± 0.90
Urea addition			no
Filter		oilfield-style filter tank	
Length of operation	days		14
Length of steady state	days		n/a

Table 2-4. Summary of normalized operating parameters and average acid concentrations for Trial 1. Error represents two standard deviations.

Trial 1		
Parameter	**Units**	**Actual**
VSLR	g VS/(L_{liq}·d)	--- ± ---
LRT	days	--- ± ---
MRT	days	--- ± ---
SRT	days	--- ± ---
TLV	L_{liq}	10,791 ± 92
NAVS conc., F1	g NAVS/L_{liq}	75.2 ± 11.5
NAVS conc., F2	g NAVS/L_{liq}	59.3 ± 7.8
NAVS conc., F3	g NAVS/L_{liq}	44.5 ± 7.11
NAVS conc., F4	g NAVS/L_{liq}	67.1 ± 10.3
Moisture content, F1	g liq./g as-is	0.876 ± 0.02
Moisture content, F2	g liq./g as-is	0.859 ± 0.04
Moisture content, F3	g liq./g as-is	0.873 ± 0.05
Moisture content, F4	g liq./g as-is	0.829 ± 0.05
Avg. ash content, F1	g ash/g dry	0.395 ± 0.05
Avg. ash content, F2	g ash/g dry	0.529 ± 0.05
Avg. ash content, F3	g ash/g dry	0.568 ± 0.05
Avg. ash content, F4	g ash/g dry	0.616 ± 0.05
pH, F1		6.1 ± 0.4
pH, F2		6.3 ± 0.4
pH, F3		6.5 ± 0.6
pH, F4		6.2 ± 0.6
Avg. acid conc., F1	g acid/L_{liq}	16.4 ± 4.7
Avg. acid conc., F2	g acid/L_{liq}	18.9 ± 4.1
Avg. acid conc., F3	g acid/L_{liq}	16.7 ± 5.2
Avg. acid conc., F4	g acid/L_{liq}	12.7 ± 7.2

2.1.4. Trial 2 (March 23–April 9, 2007)

After some trial-and-error research, it was concluded that dry solid concentration Method 4-7A (see Section 3.4.2) was sufficiently reliable to make a second attempt at steady-state operation. Because transfers had not occurred since November 13, 2006, the fermentor volumes were low (600–700 gal; set point = 800 gal). On March 19, 2007, to re-establish the set point volume and dry solid concentration, a mass balance was made for each fermentor to determine the amount of paper, chicken manure, and water that needed to be added.

On March 23, 2007, the first countercurrent transfers began for Trial 2 (second attempt at steady-state operation). Over the following three weeks, five of six scheduled transfers were executed. On March 30, 2007, the contents of Fermentor 1 were too thick to pump; thus, executing a transfer was not possible. In lieu of a transfer, to reduce the dry solid concentration, 50 gallons of unfiltered slurry was removed through the port hole and then water was added to achieve a final volume of 800 gallons. On April 12, 2007, the contents of Fermentor 1 were again unmanageably thick, indicating the material balance was flawed and not sufficiently controlling the solid concentration in Fermentor 1 so that material handling (i.e., pumping) could be maintained. The following summarizes the issues that contributed to the discontinuation of Trial 2:

- Solid concentration set points were too high. This contributed to frequent inability to pump and mix fermentor contents. Additionally, the fermentation biomass was not saturated with liquid, thereby prohibiting accurate volume balance.
- In the material balance, the moisture content, and density of transfer solids were assumed to be 0.70 g moisture/g cake and 8.5 lb/gal, respectively. These values should have been measured for each fermentor and updated frequently (each transfer or weekly).
- In the material balance, all filter liquid was assumed to be solid-free (i.e., moisture content = 1.00 g moisture/g filter liquid). In reality, the filter liquid contained 0.05–0.12 g dry solid/g filter liquid.

- The material balance did not have means to compensate/account for volume change due to digestion and liquid absorption by fresh paper.
- The gate valves on the recycle loops were fouled prohibiting fully open position.
- Sludge pumps required maintenance.
- End-of-school-semester exams limited availability of student workers. Additionally, many students were leaving town for summer causing a lapse in trained labor.

To address these issues, the mass balance calculator spreadsheet was further updated to accommodate moisture content and density data for each stream. The protocol was changed to ensure moisture content and density were measured for all streams on a regular frequency (typically each transfer). To improve pumping and volume balances, the solid concentration set points were decreased so that the biomass was fully saturated with liquid. If the biomass is not saturated, then the volume of material or liquid added does not equal the measured change in volume (e.g., added liquid may absorb into unsaturated biomass, thus no volume change is observed). Maintenance was performed. Student workers were hired and trained in preparation for Trial 3. and Tables 2-5 and 2-6 summarize the controllable and normalized operating parameters, respectively, for Trial 2.

Table 2-5. Summary of controllable operating parameters for Trial 2. Error represents two standard deviations.

Trial 2			
Parameter	**Units**	**Set point**	**Actual**
Temperature, F1	˚C	40	41.5 ± 5
Temperature, F2	˚C	40	42.6 ± 7.2
Temperature, F3	˚C	40	44.7 ± 4.4
Temperature, F4	˚C	40	44.7 ± 6.2
Transfer frequency	transfers/week	2	1.7 ± 1
paper feed rate	dry lb/transfer	100	100 ± 2
Chicken manure feed rate	dry lb/transfer	25	25 ± 1
Product transfer liquid rate, L_1	gallons/ transfer	50	50 ± 2
Total volume, F1	gallons	800	799 ± 72
Total volume, F2	gallons	800	792 ± 40
Total volume, F3	gallons	800	783 ± 20
Total volume, F4	gallons	800	770 ± 40
Dry solids conc., F1	dry lb/bulk gal	1.6–1.8	1.822 ± 0.22
Dry solids conc., F2	dry lb/bulk gal	1.9	1.942 ± 0.36
Dry solids conc., F3	dry lb/bulk gal	2.0–2.1	2.088 ± 0.20
Dry solids conc., F4	dry lb/bulk gal	2.0–2.3	2.021 ± 0.39
Urea addition			no
Filter			oilfield-style filter tank
Length of operation	days		20
Length of steady state	days		n/a

Table 2-6. Summary of normalized operating parameters and average acid concentrations for Trial 2. Error represents two standard deviations.

Trial 2				
Parameter	**Units**	**Actual**		
VSLR	g VS/(L_{liq}·d)	---	±	---
LRT	days	---	±	---
MRT	days	---	±	---
SRT	days	---	±	---
TLV	L_{liq}	9,342	±	90
NAVS conc., F1	g NAVS/L_{liq}	145.3	±	76.8
NAVS conc., F2	g NAVS/L_{liq}	157.2	±	41.3
NAVS conc., F3	g NAVS/L_{liq}	127.6	±	17.3
NAVS conc., F4	g NAVS/L_{liq}	132.7	±	39.7
Moisture content, F1	g liq./g as-is	0.796	±	0.02
Moisture content, F2	g liq./g as-is	0.787	±	0.04
Moisture content, F3	g liq./g as-is	0.777	±	0.02
Moisture content, F4	g liq./g as-is	0.784	±	0.04
Avg. ash content, F1	g ash/g dry	0.384	±	0.20
Avg. ash content, F2	g ash/g dry	0.400	±	0.10
Avg. ash content, F3	g ash/g dry	0.541	±	0.07
Avg. ash content, F4	g ash/g dry	0.525	±	0.15
pH, F1		6.3	±	0.4
pH, F2		6.3	±	0.4
pH, F3		6.2	±	0.3
pH, F4		6.4	±	0.4
Avg. acid conc., F1	g acid/L_{liq}	23.9	±	4.2
Avg. acid conc., F2	g acid/L_{liq}	20.4	±	4.1
Avg. acid conc., F3	g acid/L_{liq}	20.4	±	2.9
Avg. acid conc., F4	g acid/L_{liq}	14.2	±	6.8

2.1.5. Trial 3 – first steady state operation (May 2007 – August 2007)

On May 15, 2007, the first transfer of Trial 3 was executed. The key

operational changes that facilitated steady state were (1) reducing the solid concentration set points so biomass was saturated with liquid thereby improving volume control, (2) accounting for solids and liquids in all streams, (3) cross checking the dry solid concentration with multiple methods, and (4) using the after-mixing volume, which was a more stable value. For Trial 3, Tables 2-7 and 2-8 summarize the controllable and normalized operating parameters, respectively. See Section 4 for performance data.

Table 2-7. Summary of controllable operating parameters for Trial 3. Error represents two standard deviations.

Trial 3					
Parameter	**Units**	**Set point**	**Actual**		
Temperature, F1	˚C	40	41	±	4
Temperature, F2	˚C	40	43	±	5
Temperature, F3	˚C	40	40	±	5
Temperature, F4	˚C	---	---	±	---
Transfer frequency	transfers/week	2	1.92	±	0.03
paper feed rate	dry lb/transfer	100	100.0	±	2.0
Chicken manure feed rate	dry lb/transfer	25	25	±	1
Product transfer liquid rate, L_1	gallons/ transfer	50	50	±	2
Total volume, F1	gallons	800	760	±	101
Total volume, F2	gallons	800	791	±	31
Total volume, F3	gallons	800	801	±	43
Total volume, F4	gallons	---	---	±	---
Dry solids conc., F1	dry lb/ bulk gal	1.54	1.60	±	0.4
Dry solids conc., F2	dry lb/ bulk gal	1.93	1.91	±	0.4
Dry solids conc., F3	dry lb/ bulk gal	2.20	2.14	±	0.3
Dry solids conc., F4	dry lb/ bulk gal	---	---	±	---
Urea addition		no			
Filter		oil-field style filter tank			
Length of operation	days	84			
Length of steady state	days	53			

Table 2-8. Summary of normalized operating parameters and average steady state (SS) acid concentrations for Trial 3. Error represents two standard deviations.

Trial 3				
Parameter	**Units**	**Actual**		
VSLR	g VS/(L_{liq}·d)	1.7	±	0.1
LRT	days	166	±	7
MRT	days	93	±	5
SRT	days	126	±	2
TLV	L_{liq}	7,513	±	258
NAVS conc., F1	g NAVS/L_{liq}	112	±	32
NAVS conc., F2	g NAVS/L_{liq}	124	±	36
NAVS conc., F3	g NAVS/L_{liq}	129	±	33
NAVS conc., F4	g NAVS/L_{liq}	---	±	---
Moisture content, F1	g liq./g as-is	0.814	±	0.03
Moisture content, F2	g liq./g as-is	0.791	±	0.03
Moisture content, F3	g liq./g as-is	0.760	±	0.04
Moisture content, F4	g liq./g as-is	---	±	---
Avg. ash content, F1	g ash/g dry	0.347	±	0.12
Avg. ash content, F2	g ash/g dry	0.428	±	0.11
Avg. ash content, F3	g ash/g dry	0.503	±	0.09
Avg. ash content, F4	g ash/g dry	---	±	---
pH, F1		6.16	±	0.5
pH, F2		6.08	±	0.2
pH, F3		5.92	±	0.5
pH, F4		---	±	---
Avg. SS acid conc., F1	g acid/L_{liq}	23.9	±	4.2
Avg. SS acid conc., F2	g acid/L_{liq}	20.4	±	4.1
Avg. SS acid conc., F3	g acid/L_{liq}	20.4	±	2.9
Avg. SS acid conc., F4	g acid/L_{liq}	---	±	---

Figure 2-14. Pictures of (A) empty oilfield-style filter tank, and (B) fermented office paper and chicken manure being filtered in filter tank with gear pump used to pull vacuum via 2-inch transfer hose.

Filtration issues

Despite significant improvements in solid concentration measurement and material balance calculations, countercurrent operation remained a challenge. To separate solids and liquids for countercurrent transfer, an oilfield-style filter basket (Figure 2-14) was used. The perforated filter basket inside the filter tank had 0.25-inch diameter holes. To pull vacuum and collect filtrate, a gear pump was used as shown in Figure 2-14B. This apparatus worked well to separate fresh biomass (i.e., contents of Fermentor 1) but failed to yield good separation when the biomass particle size decreased because digestion increased (i.e., contents of Fermentors 2–4); the filter solids had a high moisture content and the filtrate had a high solid content. To quantify separation performance, the liquid recovery efficiency (100 × mass liquid in filtrate/mass liquid in slurry) and filtrate rate (filter liquid (FL) volume/time) were measured. Table 2-9 shows that the filter tank had liquid recovery efficiencies less than 50%, and filtrate

rate less than two gal FL/min, which contributed to long filtration times. Although the liquid recovery efficiency increased from Fermentors 1–3, the observed performance greatly decreased because the filtrate rate was very slow and the filtrate contained over 10% solids (i.e., moisture content = 0.89 g liquid/g total), which inflated the amount of transfer liquid and solid required by the mass balance. The large error is reflective of the inconsistent operation as a result of the following:

(A) Batch filtrations were subjectively ended based on visual observation of the "dryness" of the filter cake and if no more filtrate was generated.

(B) Slurry against the filter basket wall was dryer than slurry in basket center. To further dewater the filter cake, a shovel was used to turn over the filter cake so that wetter cake could be exposed to the strongest vacuum along the wall of the filter basket (Figure 2-14B)

(C) This procedure did increased dewatering, but was subjective and inconsistent.

(D) The strength and duration of the vacuum pulled on the filter basket was variable because of factors stated in Reason A. As the volume of cake decreased with filtration, some slurry/wet cake could get sucked through holes near the top rim of the filter basket thereby disrupting the applied vacuum.

Table 2-9. For Trial 3, solid-liquid separation performance data for material from Fermentors 1–3 separated in filter tank. Data for Fermentor 4 was not measured because it was taken offline. Error represents two standard deviations (i.e., 95% confidence interval).

	Liquid recovery efficiency	**Filtrate rate**	**Filtrate MC**	**Filter Solids MC**
	100 × (mass filtrate/ mass liquid in slurry)	gallon/min	g liquid/g total	g liquid/g total
Fermentor 1	29 ± 15 %	<2	0.97 ± 0.02	0.76 ± 0.02
Fermentor 2	37 ± 22 %	<1.5	0.94 ± 0.05	0.73 ± 0.02
Fermentor 3	48 ± 41 %	<0.5	0.89 ± 0.08	0.70 ± 0.03

MC – moisture content

As solid-liquid separation performance worsens (i.e., moisture content of filter cake and filtrate increases and decreases, respectively), to achieve the same net transfer of dry solids, the mass balance requires that greater amounts of filter cake and filtrate be transferred. The increased transfer quantities increased the time required to obtain and satisfy a mass balance. It was not uncommon for a transfer to take more than 12 hours, and occasionally, require two days to complete. Frequently, the required amounts were too great to be achieved with the available equipment (i.e., filter tank) and labor. Typically, the filtrate liquid was limiting. When this occurred, operation was forced to deviate from the mass balance (e.g., add fresh water to Fermentor 2).

Strategies for coping with forced deviation from material balance

To obtain “clean” liquid (i.e., minimal solids; >0.97 g moisture/g total) and help satisfy the mass balance, extra filter baskets were shoved into the biomass (Figure 2-15A) either the night prior to transfer and/or while other tasks were being completed. Because of the modest hydrostatic pressure, the basket would slowly fill with liquid. Because of the success this technique, a longer filter basket (48” long) was fabricated to increase the amount of percolated liquid that could be collected (Figure 2-15B). This percolation technique could be used to obtain varying amounts (5–200 gallons) of clean liquid depending on the size of the basket(s) used, solid concentration, time, and particle size. The liquid was removed from the filter baskets using a submersible pump and/or manually with a cup. This filtration technique is referred to as the Percolation method.

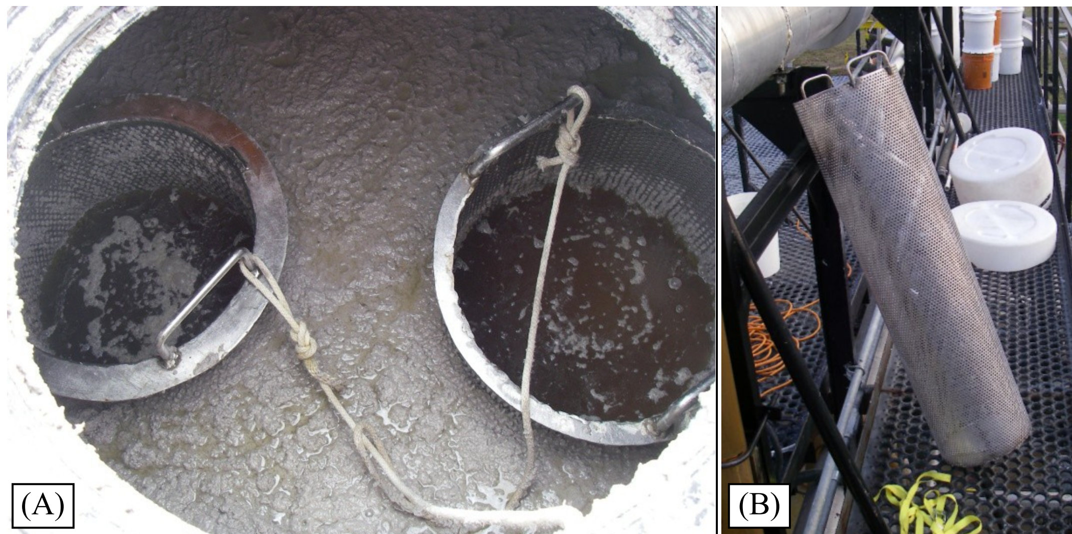


Figure 2-15. Pictures of (A) small filter baskets placed in fermentor to obtain extra liquid and (B) long (48”) filter basket used to maximize collection of percolated liquid.

If insufficient liquid was obtain (both filtrate and percolation liquid) to satisfy the mass balance, to mitigate the forced deviation from the mass balance, liquid was “leap frogged” from a subsequent fermentor. For example, the mass balance dictated that 150 gallons of liquid be transferred from Fermentor 3 to Fermentor 2. However, only 100 gallons of liquid was obtained from the filtration device and Percolation method; thus, to achieve the target volume and solid concentration, 50 gallons of liquid is need. Because Fermentor 3 was 50 gallons of liquid short, the amount of liquid transferred from Fermentor 4 to Fermentor 3 is reduced by 50 gallons. Rather than add fresh water (last resort), the incremental 50 gallons of liquid that should have been transferred from Fermentor 4 to 3 is transferred from Fermentor 4 to 2. This “leap frog” technique helps maintain the acid concentration in Fermentor 2, whereas addition of fresh water would further dilute the acid concentration. If there is insufficient liquid obtained from Fermentor 4 to use this technique, then fresh water would be added as a final option. As the system approached steady state, 50% of the transfers had forced deviations from the mass balance due to equipment and labor limitations.

Pump issues

A few weeks after Trial 3 began, Fermentor 4 sludge pump began to have problems. The pump would discharge biomass out the bottom port (forward operation, shortest path) but would not suck liquid (reverse operation), nor recycle well (forward operation, longest path). By the middle of June, Fermentor 4 sludge pump failed to move slurry in either direction. The failure was because the stator and rotor had been damaged by hard particles (e.g., metal paper clips; see page 26 and Figure 2-12) in the fermentation. Because it took a long time to obtain and install replacement parts, it was decided to only operate with three fermentors. Further, because of difficulties with solid-liquid separation and time required to complete a transfer, continued operation with four fermentors was not practical.

Screwpress needed

From the beginning of Trial 3 it was clear that the oilfield-style filter tank was not an ideal solid-liquid separation device; however, limited resources and insufficient data constrained operation to its use. By August, steady-state operation had been achieved (or at least a very close approximation). It was clear that the strategies used to operate the pilot fermentation at steady state were robust; however, the operation suffered (i.e., unable to complete transfer according to mass balance) from inadequate equipment and dramatically increased labor cost. After review of solid-liquid separation devices, it was determined that a screwpress was the most appropriate device to replace the filter tank. A request was made to Terrabon Inc. (industry partner) for funding to rent a KP-6 screwpress from Vincent Corporation, which was granted. After the August 6, 2007 transfer, countercurrent operation of the pilot fermentation was suspended while a screwpress was obtained and installed. The fermentors were maintained in batch mode (i.e., fermentors monitored (pH, temp, volume, solid concentration) and inhibitor added).

2.1.6. Trial 4; second steady-state operation (October 2007– December 2007)

By the end of September 2007, the screwpress (Figure 2-16) had been installed

and tested. On October 9, 2007, Trial 4 began with paper, chicken manure, and fresh water added to each fermentor to reestablish the set point volume and solid concentration. The first transfer was executed on October 12th. By early November, the beginning of steady-state acid concentration was observed. Countercurrent operation continued through December 20th it was determined that sufficient steady-state data had been obtained. For Trial 4, Tables 2-10 and 2-11 summarize the controllable and normalized operating parameters, respectively. See Section 4 for performance data.

Screwpress

As expected, the screwpress separated solids and liquid extremely well; the liquid contained 4–7% solids (0.93–0.96 g liquid/g total) and the solids had less than 0.6 g liquid/g total. The screwpress could be run continuously, whereas the oilfield-style filter tank was batch-wise operation. The screwpress generated filter liquid 5–10 times faster than could be achieved with the filter tank. The increased performance reduced the time required to complete a transfer by about half.

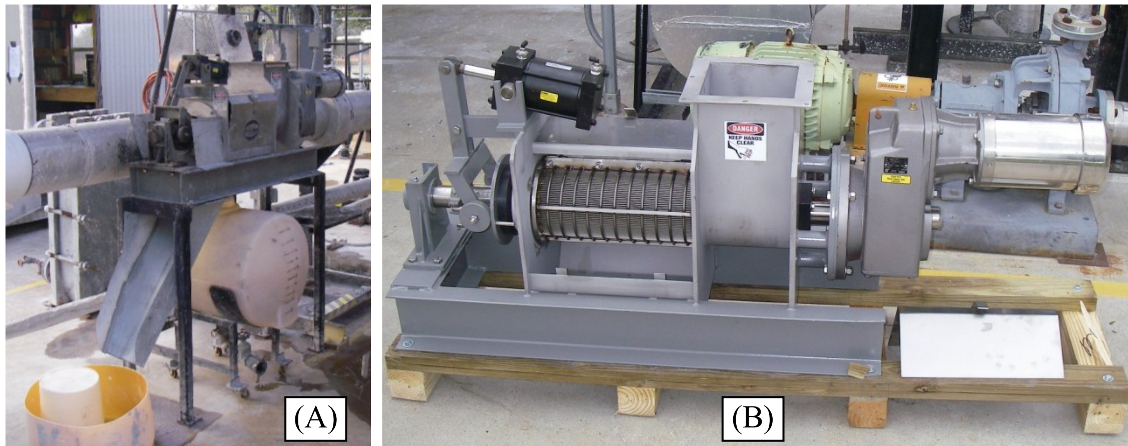


Figure 2-16. Pictures of Vincent KP-6 screwpress (A) mounted on stand and (B) new.

Table 2-10. Summary of controllable operating parameters for Trial 4. Error represents two standard deviations.

Trial 4					
Parameter	**Units**	**Set point**	**Actual**		
Temperature, F1	˚C	40	38	±	6
Temperature, F2	˚C	40	39	±	9
Temperature, F3	˚C	40	37	±	7
Temperature, F4	˚C	---	---	±	---
Transfer frequency	transfers/week	2	1.82	±	0.1
paper feed rate	dry lb/transfer	100	100	±	2
Chicken manure feed rate	dry lb/transfer	25	25	±	1
Product transfer liquid rate, L_1	gallons/ transfer	50	50	±	2
Total volume, F1	gallons	800	804	±	49
Total volume, F2	gallons	800	789	±	42
Total volume, F3	gallons	800	798	±	40
Total volume, F4	gallons	---	---	±	---
Dry solids conc., F1	dry lb/ bulk gal	1.50	1.46	±	0.5
Dry solids conc., F2	dry lb/ bulk gal	1.83	1.80	±	1.0
Dry solids conc., F3	dry lb/ bulk gal	1.95	1.91	±	1.2
Dry solids conc., F4	dry lb/ bulk gal	---	---	±	---
Urea addition			no		
Filter			Vincent KP-6 screw press		
Length of operation	days		73		
Length of steady state	days		58		

Table 2-11. Summary of normalized operating parameters and average steady-state (SS) acid concentrations for Trial 4. Error represents two standard deviations.

Trial 4				
Parameter	**Units**	**Actual**		
VSLR	g VS/(L_{liq}·d)	1.5	±	0.1
LRT	days	182	±	7
MRT	days	95	±	5
SRT	days	126	±	4
TLV	L_{liq}	7,719	±	258
NAVS conc., F1	g NAVS/L_{liq}	116	±	20
NAVS conc., F2	g NAVS/L_{liq}	164	±	43
NAVS conc., F3	g NAVS/L_{liq}	157	±	43
NAVS conc., F4	g NAVS/L_{liq}	---	±	---
Moisture content, F1	g liq./g as-is	0.822	±	0.05
Moisture content, F2	g liq./g as-is	0.796	±	0.11
Moisture content, F3	g liq./g as-is	0.783	±	0.15
Moisture content, F4	g liq./g as-is	---	±	---
Avg. ash content, F1	g ash/g dry	0.288	±	0.08
Avg. ash content, F2	g ash/g dry	0.339	±	0.10
Avg. ash content, F3	g ash/g dry	0.393	±	0.09
Avg. ash content, F4	g ash/g dry	---	±	---
pH, F1		6.11	±	0.4
pH, F2		6.01	±	0.4
pH, F3		6.02	±	0.6
pH, F4		---	±	---
Avg. SS acid conc., F1	g acid/L_{liq}	29.30	±	1.80
Avg. SS acid conc., F2	g acid/L_{liq}	21.69	±	3.07
Avg. SS acid conc., F3	g acid/L_{liq}	14.83	±	4.20
Avg. SS acid conc., F4	g acid/L_{liq}	---	±	---

Compared to Trial 3, Trial 4 was much less physically demanding, required fewer man-hours, mass balances were satisfied as calculated, and had less challenging operational issues, which were the (1) separation rate of material from Fermentor 3 and (2) pump failure (see Section 2.2.1). Table 2-12 shows that the liquid recovery

efficiency and filtrate rate decreased from Fermentor 1 to 3. Compared to Trial 3 (liquid recovery efficiency 20–50%), the liquid recovery efficiency was much higher in Trial 4 and the moisture content of the filter solids and filtrate were more consistent, which improved the precision and accuracy of the material balances. Separation performance decreased from Fermentor 1 to 3 because increased digestion decreased particle size. The screwpress worked best with fibrous material (Fermentor 1), which is more readily squeezed. It performed the worst with highly digested material. When more digested slurry is compressed, the smaller more spherical particles slide past one another more easily than more fibrous particles, thereby reducing the compression needed to purge the absorbed liquid.

The main challenge encountered using the screwpress was the slow filtrate rate of material from Fermentor 3. Because, typically, disproportionally more filter liquid (FL) was needed from filtration than filter cake (i.e., filter liquid was the limiting filtration product) and because measuring the liquid collection rate was straightforward, the filtration rate is measured with respect to the rate filter liquid is generated. Typically, the filtration rate for slurry from Fermentor 3 was 0.5–1.0 gal FL/min. However, as steady state was established, the filtrate rate decreased to <0.5 gal FL/min. Occasionally, it was as low as 0.1 gal FL/min. Over the duration of Trial 4, the decreased filtration rate increased the time required to obtain the target liquid volume from Fermentor 3 by a factor of 5–10. To increase the filtration rate, fresh paper was used as a filter aid; paper was combined with material from Fermentor 3 in the screwpress hopper. Use of paper as a filter aid maintained the filtration rate of material from Fermentor 3 >0.5 gal FL/min, which was slow but manageable.

The use of filter aid is not ideal, especially if filter solids, which contains the filter aid, are returned to Fermentor 3. Because Fermentor 3 was the last stage, filter solids removed were waste; thus, filter aid in the waste did not affect the active fermentation. For the research purposes of this pilot fermentation, use of a filter aid was acceptable. However, in an industrial setting, this may not be feasible. From this experience, it is apparent that (1) a separation device is needed that has acceptable

performance with both fibrous and fine particles or (2) an additional devices is needed that separates slurry with fine particles rapidly and efficiently.

Table 2-12. Trial 4 steady-state liquid recovery efficiency and filtration rate for material from Fermentors 1–3 separated using a Vincent KP-6 screwpress. Error represents two standard deviations.

	Liquid recovery efficiency	**Filtration rate**	**Filtrate MC**	**Filter Solids MC**
	100 × (mass filtrate/ mass liquid in slurry)	gal FL/min	g liquid/g total	g liquid/g total
Fermentor 1	83 ± 4 %	~5	0.96 ± 0.01	0.50 ± 0.03
Fermentor 2	70 ± 5 %	2–3	0.96 ± 0.01	0.59 ± 0.03
Fermentor 3	56 ± 8 %	<1	0.95 ± 0.04	0.66 ± 0.04

MC – moisture content

2.1.7. Trial 5; urea addition (January–March 2008)

The success observed with Trials 3 and 4 demonstrated control and validated the methods and techniques used to achieve steady state. Now that is was understood how to achieve steady state, the goal of Trial 5 was to increase performance (i.e., yield, conversion, selectivity, productivity) by adding urea as a nitrogen supplement. Although Trial 5 did not result in steady-state operation, meaningful hard-learned lessons were made. The following subsections describe aspects of operation and discuss related lessons learned. For Trial 5, Tables 2-13 and 2-14 summarize the controllable and normalized operating parameters, respectively.

Fermentor dilution

Based on the filtration rate issues observed during Trial 4, it was decided that Trial 5 would be initiated with fresh fibrous biomass added to each fermentor, which

would improve filtration rates. On January 12, 2008, bulk material was removed from each fermentor until a volume of 650 gallons was achieved. Then, 100 lb paper and 25 lb chicken manure were added on a dry basis. Water was added until the final volume was 800 gallons. The first transfer was executed on January 18th.

Table 2-13. Summary of controllable operating parameters for Trial 5. Error represents two standard deviations.

Trial 5					
Parameter	**Units**	**Set point**	**Actual**		
Temperature, F1	˚C	40	39.2	±	7.04
Temperature, F2	˚C	40	36.8	±	6.3
Temperature, F3	˚C	40	39.6	±	7.94
Temperature, F4	˚C	---	---	±	---
Transfer frequency	transfers/week	2	1.99	±	0.1
paper feed rate	dry lb/transfer	100	100	±	2
Chicken manure feed rate	dry lb/transfer	25	25	±	1
Product transfer liquid rate, L_1	gallons/ transfer	50	50	±	2
Total volume, F1	gallons	800	810	±	51
Total volume, F2	gallons	800	791	±	46
Total volume, F3	gallons	800	799	±	45
Total volume, F4	gallons	---	---	±	---
Dry solids conc., F1	dry lb/ bulk gal	1.50	1.43	±	0.3
Dry solids conc., F2	dry lb/ bulk gal	1.75	1.73	±	0.3
Dry solids conc., F3	dry lb/ bulk gal	1.85	1.80	±	0.3
Dry solids conc., F4	dry lb/ bulk gal	---	---	±	---
Urea addition		yes			
Filter		Vincent KP-6 screw press			
Length of operation	days	60			
Length of steady state	days	n/a			

Table 2-14. Summary of normalized operating parameters and average acid concentrations for Trial 5. Error represents two standard deviations.

Trial 5				
Parameter	**Units**	**Actual**		
VSLR	g VS/(L_{liq}·d)	1.6	±	0.1
LRT	days	171	±	9
MRT	days	94	±	8
SRT	days	113	±	3
TLV	L_{liq}	7,836	±	214
NAVS conc., F1	g NAVS/L_{liq}	124	±	19
NAVS conc., F2	g NAVS/L_{liq}	157	±	11
NAVS conc., F3	g NAVS/L_{liq}	163	±	33
NAVS conc., F4	g NAVS/L_{liq}	---	±	---
Moisture content, F1	g liq./g as-is	0.844	±	0.02
Moisture content, F2	g liq./g as-is	0.810	±	0.02
Moisture content, F3	g liq./g as-is	0.806	±	0.02
Moisture content, F4	g liq./g as-is	---	±	---
Avg. ash content, F1	g ash/g dry	0.260	±	0.03
Avg. ash content, F2	g ash/g dry	0.312	±	0.02
Avg. ash content, F3	g ash/g dry	0.366	±	0.04
Avg. ash content, F4	g ash/g dry	---	±	---
pH, F1		6.34	±	0.6
pH, F2		6.01	±	0.8
pH, F3		6.09	±	1.0
pH, F4		---	±	---
Avg. acid conc., F1	g acid/L_{liq}	24.1	±	8.6
Avg. acid conc., F2	g acid/L_{liq}	20.5	±	5.2
Avg. acid conc., F3	g acid/L_{liq}	13.6	±	8.3
Avg. acid conc., F4	g acid/L_{liq}	---	±	---

In hindsight, the addition of fresh substrate and water to each fermentor was a mistake for several reasons. (1) Water addition to each fermentor dramatically reduced the acid concentration in each fermentor that led to a long delay. From the product liquid acid data shown in Figure 2-17, it took approximately 40 days for the acid

concentration to stabilize. If the fermentation was not diluted, acid concentration would have likely stabilized within a few weeks. (2) Addition of fresh substrate to Fermentors 2 and 3 artificially adds confusion to performance calculations, which assumes all fresh substrate is fed to Fermentor 1. (3) The filtering benefit of fresh fibrous substrate added to each fermentor was short-lived and did not represent steady-state behavior. In retrospect, it would have been better to have coped with the slow filtration while investigating techniques and/or equipment that would have helped overcome the problem.

On January 31, 2008, the contents of Fermentor 1 were very thick and would not pump. The following day was the next transfer. If the slurry cannot be pumped, then executing a transfer is not possible. To alleviate this problem, 80 gallons of fresh water was added. This action was not ideal but was necessary to continue operation. The added fresh water diluted the product liquid acid concentration, which added approximately a one-week delay (Figure 2-17). The cause of Fermentor 1 constipation was believed to result from biased solid concentration data. The employed sample technique only collected slurry from the bottom port of the recycle loop. The fresh biomass in Fermentor 1 would trap gas bubbles produced during fermentation and float. This phenomenon caused the dry solid concentration on the bottom of the fermentor, where the sample was collected, to be lower than the whole.

Urea addition

Previous works had investigated urea addition in batch fermentations and had supplemented countercurrent fermentations with urea; however, this literature only provided precedent rather than quantitative guidance on how to determine the appropriate urea addition rate (Agbogbo and Holtzapple, 2007; Aiello-Mazzarri et al., 2006; Chan, 2002; Coleman, 2007; Domke et al., 2004; Thanakoses et al., 2003).

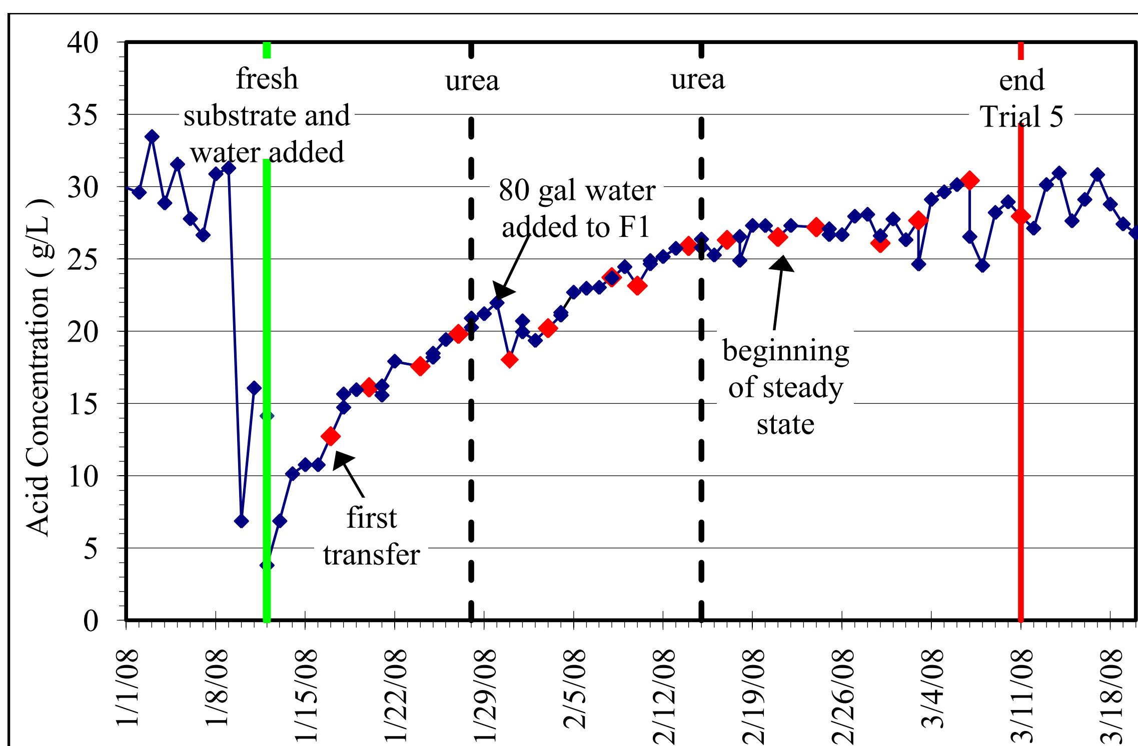


Figure 2-17. Trial 5 product liquid acid concentration with event markers. Blue data points are daily acid concentration. Red data points are transfer day acid concentration.

To guide nitrogen supplementation, an optimal carbon-nitrogen ratio of 30 g carbon/g nitrogen (not g non-acid carbon/g nitrogen as is used in Sections 6–8) was assumed. During Trial 4, a steady-state slurry C/N ratio of 30, 47, and 56 g carbon/g nitrogen was measured in Fermentors 1–3, respectively. This C/N ratio profile indicated the net direction of nitrogen transport was with the liquid stream. To prevent the C/N ratio in Fermentor 1 from becoming too low, it was decided that urea would be added to Fermentors 2 and 3 or only Fermentor 3; the urea would be distributed to Fermentors 1 and 2 via liquid transfer.

On January 10th and 22nd, the carbon and nitrogen content of each fermentor was measured. Total C and N contents (g/100 g as-is sample) were determined by Texas A&M University Soil, Water and Forage Testing Lab (College Station, TX) using a Elementor Variomax CN. Based on the average C and N contents, the amount of urea

that needed to be added to each fermentor to achieve a final C/N of 30 g carbon/g nitrogen was calculated. For Fermentors 1–3, 13, 22, and 24 lb of urea were required, respectively. Based on the above-stated urea addition strategy and rounding down to be conservative (avoid ammonia toxicity and/or basic pH), 20 lb of urea was added to Fermentors 2 and 3 during the January 28th transfer. Using this process, urea was added again on February 15th (15 lb urea to Fermentor 3).

Figure 2-18 shows the effect urea had on fermentor C/N ratio; the C/N ratio in the fermentors that received urea significantly decreased after urea was added. The C/N ratio of Fermentor 1 decreased with respect to time indicating urea transport with liquid streams. Between January 29th and February 2nd, the C/N ratio in Fermentor 1 increased. This is an illogical trend and is believed to result from erroneous data. The following two issues that make controlling the fermentation C/N ratio challenging: (1) a small quantity (<1 g) of material is used for C and N analysis; thus, data may or may not be representative of the entire fermentation. (2) Because analysis was outsourced, it could take several weeks to receive data; thus, urea addition was based on data that was 1–3 weeks old. The control frequency was limited by this time delay.

Filtration rate

Because steady state was not achieved, it is unknown how urea influenced performance (yield, conversion, selectivity, productivity). However, qualitatively, the urea appeared to increase digestion (i.e., substrate was more decomposed; smaller particle size). Unfortunately, the increased digestion exacerbated the slow filtration rate issue discussed in Trial 4. As shown in Table 2-15, the filtration rate was typically <0.2 gal FL/min and frequently <0.1 gal FL/min. Such slow rates significantly increased the time required to complete the transfer. Relative to the amount of filter liquid and filter solids produced by the screwpress, typically more filter liquid was need than filter solids; thus, there was more emphasis on filter liquid rate (gal FL/min) rather than filter solids rate. Despite using filter baskets to obtain extra liquid (described in Trial 3) and using paper as a filter aid, the time required to complete a transfer became excessively long.

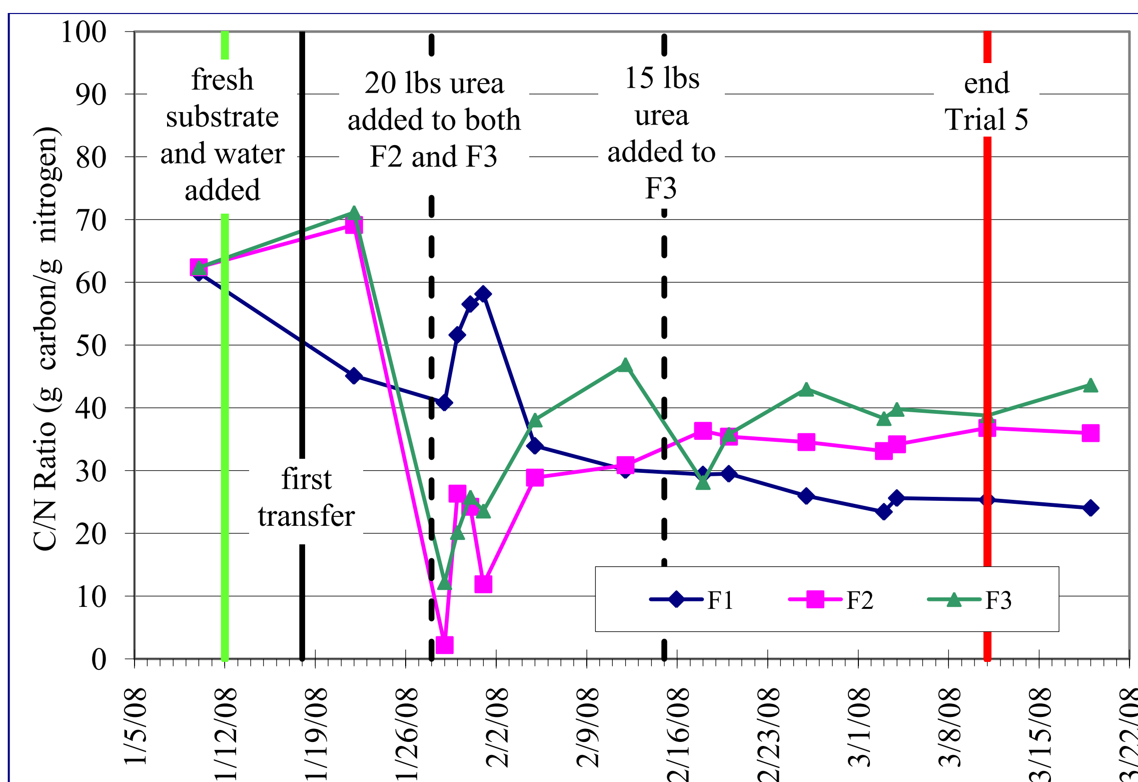


Figure 2-18. C/N ratio of Fermentors 1–3 during Trial 5 with event markers.

Table 2-15. Trial 5 average liquid recovery efficiency and filtration rate for material from Fermentors 1–3 separated using a Vincent KP-6 screwpress. Error represents two standard deviations.

	Liquid recovery efficiency	Filtration rate	Filtrate MC	Filter Solids MC
	100 × (mass filtrate/ mass liquid in slurry)	gal FL/min	g liquid/g total	g liquid/g total
Fermentor 1	82 ± 7 %	~5	0.97 ± 0.02	0.52 ± 0.05
Fermentor 2	62 ± 14 %	~2	0.95 ± 0.02	0.64 ± 0.05
Fermentor 3	53 ± 25 %	<0.2	0.94 ± 0.03	0.66 ± 0.06

MC – moisture content

For example, consider the scenario that occurred on March 7, 2008. The liquid demand from Fermentor 3 was 95 gallons. From the filter baskets using the percolation method, 15 gallons of liquid were obtained and 9 gallons of filtrate had been saved from the previous day's solid concentration measurement; thus, 72 gallons of filter liquid was needed. The filtration rate for slurry from Fermentor 3 was 5 gallons FL *per hour* (i.e., ~0.8 gal FL/min). At that rate, it would have taken 14.5 hours (i.e., until 4:00 am the following morning) to obtain the target amount of filtrate. To avoid this excessive time requirement, filtration was stopped when 22 gallons were obtained. To make up the remaining 50 gallons needed to transfer to Fermentor 2, 25 gallons of product liquid was mixed with 25 gallons of water, which approximated the acid concentration of filtrate from Fermentor 3. Because of the excessively slow filtration rates, on March 11th, Trial 5 was ended. A new strategy, technique, and/or solid-liquid separation equipment was needed that could filter highly digested material and decrease the labor requirement.

2.1.8. Trial 6 (May–October 2008)

Filtration strategy

After Trial 5 was ended, equipment was investigated that could filter slurries containing fine particles. A filter press and a decanter centrifuge were identified; however, it was learned that neither device would work well with the high solids content (>0.10 g dry solids/g total) fermentations used at the pilot plant. Additionally, funds were limited to purchase or rent another piece of equipment.

The Percolation method described in Trial 3 worked well to collect liquid. However, because the fermentation had a high solid content (~0.14 g dry solid/g total), this technique often produced only a modest amount of liquid (5–30 gallons) and was also slow (percolation occurred over night). From experience, it was known that at lower solid concentrations, the solids would settle thereby creating a top layer of free liquid and increasing the amount of liquid that could be obtained via the Percolation method.

To overcome filtration-rate issues discussed in Trials 4 and 5 without purchase or rent of additional equipment, the Percolation method was used to filter Fermentor 3. All

liquid that could be obtained via the Percolation method would be removed and used as filtrate. The solid-rich slurry that remained was used as filter solids. Over the course of Trial 6, the solid concentration was lowered so that sufficient liquid could be obtained to satisfy the mass balance. The screwpress was used to filter Fermentors 2 and 3.

The goal of Trial 5 was to quantify the benefit of nitrogen supplementation (urea addition) by comparison to Trial 4. Because the Trial 6 filtration strategy is significantly different than that used in Trial 4, the benefit of nitrogen supplementation in Trial 6 could not be meaningfully discerned by comparison to Trial 4. To make a fair comparison, the goal was to use the new filtration strategy to achieve steady state without urea addition. Then, using the same filtration strategy, a subsequent steady state would be achieved with urea addition.

The first transfer of Trial 6 was executed on May 19, 2008. Because of the experience gained from Trials 1–5, the day-to-day operations were the most consistent during Trial 6. Because of past refinements, there was greater precision with respect to volume and dry solid concentration measurements. Additionally, during Trial 6, solid concentration measurement techniques 4-2B and 4-9A (see Section 3.4.2 for more details) were further refined. Because of improvements in measurements and increased accounting, material balances were more accurate and better controlled the volume and solid concentration in each fermentor. As a result, the amount of filtrate and filter solids required each transfer was more consistent. Except for a few initial transfers, all transfers were executed according to the material balance calculations. Tables 2-16 and 2-17 summarize the controllable and normalized operating parameters, respectively.

Table 2-16. Summary of controllable operating parameters for Trial 6. Error represents two standard deviations.

Trial 6					
Parameter	**Units**	**Set point**	**Actual**		
Temperature, F1	˚C	40	43.1	±	6.02
Temperature, F2	˚C	40	44.9	±	8.67
Temperature, F3	˚C	40	39.5	±	11.8
Temperature, F4	˚C	---	---	±	
Transfer frequency	transfers/week	2	1.87	±	0.2
paper feed rate	dry lb/transfer	100	100	±	2
Chicken manure feed rate	dry lb/transfer	25	25	±	1
Product transfer liquid rate, L_1	gallons/ transfer	50	50	±	2
Total volume, F1	gallons	800	809	±	40
Total volume, F2	gallons	800	792	±	39
Total volume, F3	gallons	800	795	±	143
Total volume, F4	gallons	---	---	±	
Dry solids conc., F1	dry lb/ bulk gal	1.50	1.48	±	0.1
Dry solids conc., F2	dry lb/ bulk gal	1.42	1.42	±	0.2
Dry solids conc., F3	dry lb/ bulk gal	1.14	1.16	±	0.4
Dry solids conc., F4	dry lb/ bulk gal	---	---	±	
Urea addition			no		
Filter	Vincent KP-6 screw press (F1 & F2), Percolation method (F3)				
Length of operation	days		139		
Length of steady state	days		n/a		

Table 2-17. Summary of normalized operating parameters and average acid concentrations for Trial 6. Error represents two standard deviations.

Trial 6				
Parameter	**Units**	**Actual**		
VSLR	g VS/(L_{liq}·d)	1.12	±	0.2
LRT	days	201	±	14
MRT	days	147	±	12
SRT	days	121	±	18
TLV	L_{liq}	7,965	±	276
NAVS conc., F1	g NAVS/L_{liq}	128	±	18
NAVS conc., F2	g NAVS/L_{liq}	136	±	19
NAVS conc., F3	g NAVS/L_{liq}	108	±	12
NAVS conc., F4	g NAVS/L_{liq}	---	±	---
Moisture content, F1	g liq./g as-is	0.831	±	0.01
Moisture content, F2	g liq./g as-is	0.839	±	0.01
Moisture content, F3	g liq./g as-is	0.866	±	0.02
Moisture content, F4	g liq./g as-is	---	±	---
Avg. ash content, F1	g ash/g dry	0.238	±	0.05
Avg. ash content, F2	g ash/g dry	0.261	±	0.12
Avg. ash content, F3	g ash/g dry	0.211	±	0.05
Avg. ash content, F4	g ash/g dry	---	±	
pH, F1		6.52	±	0.7
pH, F2		6.18	±	1.2
pH, F3		6.25	±	0.6
pH, F4		---	±	---
Avg. acid conc., F1	g acid/L_{liq}	23.5	±	8.64
Avg. acid conc., F2	g acid/L_{liq}	13.1	±	6.78
Avg. acid conc., F3	g acid/L_{liq}	6.0	±	5.48
Avg. acid conc., F4	g acid/L_{liq}	---	±	---

Diluted acid concentration

Although the Percolation method is a simple and easy way to "filter" the fermentation, the effective separation efficiency is low and the "filter solids" have high moisture content. To compensate for the large amount of liquid lost with the waste filter solids, more fresh water was added according to the material balance. The increased water fed to Fermentor 3 caused the acid concentration in all fermentors to decrease with respect to time (Figure 2-19).

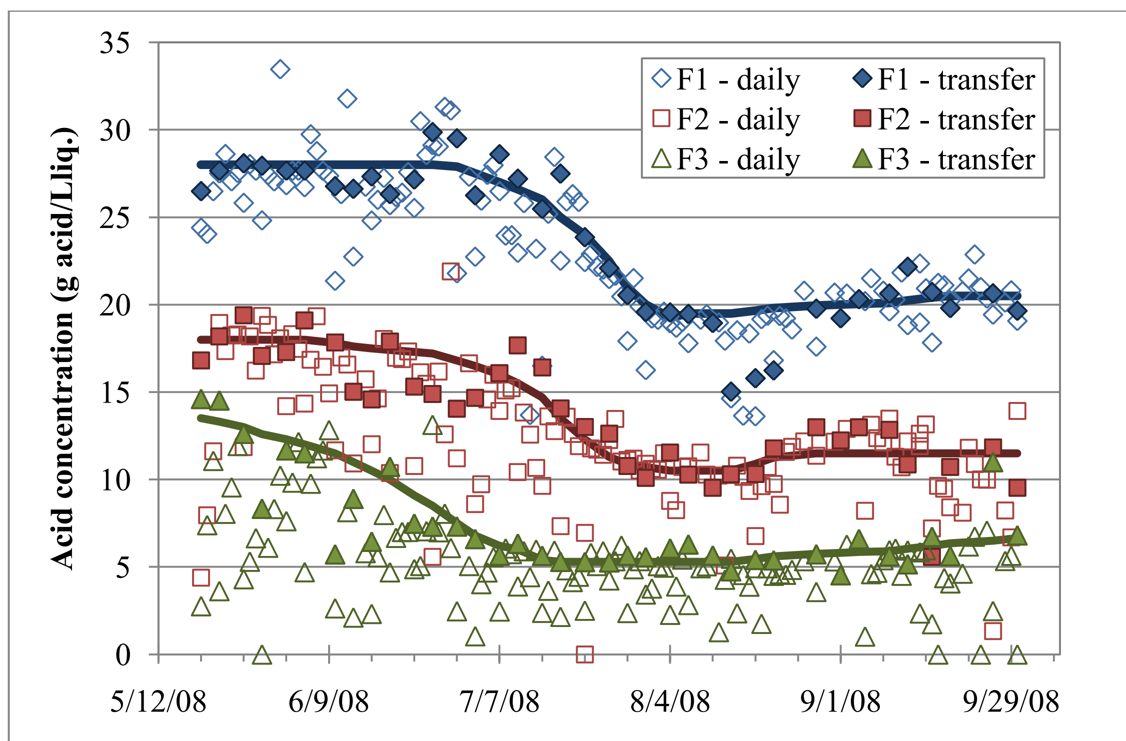


Figure 2-19. For Trial 6, daily and transfer day acid concentration in Fermentors 1–3 with hand-drawn trend lines.

During Trial 6 as acid data were collected, the trends shown in Figure 2-19 were not apparent because the original data (i.e., not data shown in Figure 2-19) showed dramatic step changes in acid concentration and other irrational trends, which were

believed to be a result of temporal issues with the gas chromatograph and/or sample preparation made. Despite this issue, transfers were persisted in hope that reanalysis of data would show steady state was achieved. To eliminate possible errors from gas chromatography, the samples were reanalyzed after Trial 6 had ended. Figure 2-19 shows the reanalyzed acid data from Trial 6. Although a dilution wave propagated through the fermentation and there is noise in the data, it appears quasi-steady state was achieved.

Filtration performance

Compared to Trial 4, the solid-liquid separation performance of the screwpress during Trial 6 was similar (Table 2-18). The liquid recovery efficiency and filtration rate for material in Fermentor 3 was higher in Trial 6 than Trial 4 because the dry solid concentration was lower during Trial 6; thus, there was more free liquid (liquid not absorbed in solid), which was more readily separated. As expected, the liquid recovery efficiency of the Percolation method was much lower than the screwpress (50–80%) or oilfield-style filter basket (30–50%). The filtration rate of the Percolation method was inversely related to the dry solid concentration.

Although the filtration strategy did work, it was not ideal. The primary disadvantage of the Percolation method is the filter solids have a moisture content just slightly lower than the bulk slurry. This is problematic because (1) of the dilution effect (discussed above) and (2) a large amount of liquid, which contains acid product, is discarded when waste solids are discarded. Ideally, the waste solids should have a low moisture content and be washed with the fresh waster feed to recover acid in the waste.

Table 2-18. Trial 6 average liquid recovery efficiency and filtration rate for material from Fermentors 1–3 separated using a Vincent KP-6 screwpress and Fermentor 3 separated by Percolation method (PM). Error represents two standard deviations.

	Liquid recovery efficiency	**Filtration rate**	**Filtrate MC**	**Filter Solids MC**
	100 × (mass filtrate/ mass liquid in slurry)	gal FL/min	g liquid/g total	g liquid/g total
Fermentor 1	79 ± 6%	~5	0.96 ± 0.02	0.50 ± 0.06
Fermentor 2	76 ± 11%	2–3	0.97 ± 0.03	0.54 ± 0.10
Fermentor 3	65 ± 26%	~1	0.96 ± 0.06	0.64 ± 0.09
Fermentor 3^{PM}	15 ± 8%	1–5	0.98 ± 0.02	0.83 ± 0.05

MC – moisture content

Pilot fermentation suspended

The primary goal of the pilot fermentation was to achieve steady state so that performance could be compared with CPDM, which was accomplished during Trials 3 and 4. The focus of Trials 5 and 6 were to increase performance through nitrogen supplementation, which would have helped promote the technology towards commercial development.

During Trials 5 and 6, many questions surfaced regarding urea addition and the control of nitrogen in a countercurrent stage fermentation. How much urea should be added during each transfer? What is the optimum C/N ratio for mixed-acid fermentation? To which fermentors should the urea be added? How does nitrogen move in the system? Can this behavior be controlled and/or modeled? In trying to answer these basic questions, it became obvious that little was understood about nitrogen in a countercurrent staged mixed-acid fermentation. To answer these questions, the experiments in Sections 6–8 were proposed.

Without understanding how to control the nitrogen content and/or C/N ratio of a countercurrent staged fermentation, over-addition of urea could occur and a steady state

nitrogen and/or C/N ratio would likely occur. To understand these issues, laboratory experiments are more appropriate and much less expensive. Further, without equipment that can separate highly digested slurry, additional attempts to increase pilot fermentation performance could likely result in failure (i.e., steady state not achieved, deviation from material balance) and the time, effort, and expense would be wasted. For these reasons, the pilot fermentation was suspended and laboratory fermentations were started to investigate the above-mentioned questions.

2.2. Solids handling and equipment issues

2.2.1. Material handling

Material handling issues were a regular problem that caused unanticipated maintenance, delays, increased cost, and increased labor requirement. For the pilot fermentation, material handling issues were related to one of four categories: (1) sludge pumps, (2) valves, (3) solid handling, and (4) solid-liquid separation equipment, which was discussed previously in Sections 2.1.5–8. The following subsections discuss material handling Categories 1–3.

Sludge pumps

The bottom cone of each fermentor fed biomass to a Moyno progressive-cavity sludge pump that moved the biomass slurry through the recycle loop or transfer hose and could suck liquid into the fermentor from a measuring tank. The primary problem with these pumps is they were easily damaged by hard objects (e.g., metal paper clips; see page 26 and Figure 2-12). Progressive-cavity pumps rely on a tight seal between the hard rubber stator and the metal rotor. Hard abrasive objects can damage both the stator and rotor (Figure 2-20). Over time, the seal is compromised. Pump performance decreases until the pump no longer functions

Although the office paper supply was changed to a less object-prone source and procedures were implemented to screen out objects, hard particles would occasionally get in the fermentation. It was also suspected that sand from the original inocula

contributed to stator abrasion. Between start-up (September 2006) and the end of Trial 6 (October 2008), ten stators and four rotors were replaced (Figure 2-20). These parts were expensive (stator ~$365, rotor ~$820) and time-consuming to replace. Because progressive-cavity pumps are easily damaged by hard objects and particles, which will inevitably be present in waste feedstock, future pilot and commercial operations should use more robust pumps that are less easily damaged.

These pumps commonly leaked liquid and occasionally biomass (Figure 2-21). For these types of pumps, slow drips (e.g., one drip every 10 minutes) are necessary to keep the packing moist. However, sometimes the leaks could be almost continuous amounting to 5–15 gallons of liquid lost per day. To control the leaks, the packing had to be tightened as needed and periodically replaced.

When the pumps were working properly, they could be overwhelmed if the solid concentration got too high. The pumping limit depends on the particle size and the current condition of the working parts (i.e., stator and rotor). The exact limit was not determined; however, the solid concentration set points used in Trial 2 are an approximation. To ensure the fermentation can be pumped, when calculating mass balances, it is better that the final solid concentration be lower than higher (i.e., better to error on remove more solids than less). If the fermentor becomes too thick to pump, the only options are to (1) wait for it to digest and (2) dilute with water or liquid from another fermentor.

Valves

During Trial 2, the 3-inch gate valve on the recycle loop had clogged. Once the valve was removed and inspected, it was observed that the valves had biomass behind the gate, which prevented the valve from being fully opened (Figure 2-22). To prevent future clogging, the three-inch gate valves were removed from all fermentor recycle loops and replaced with 3-inch plastic ball valves, which are less prone to fouling.

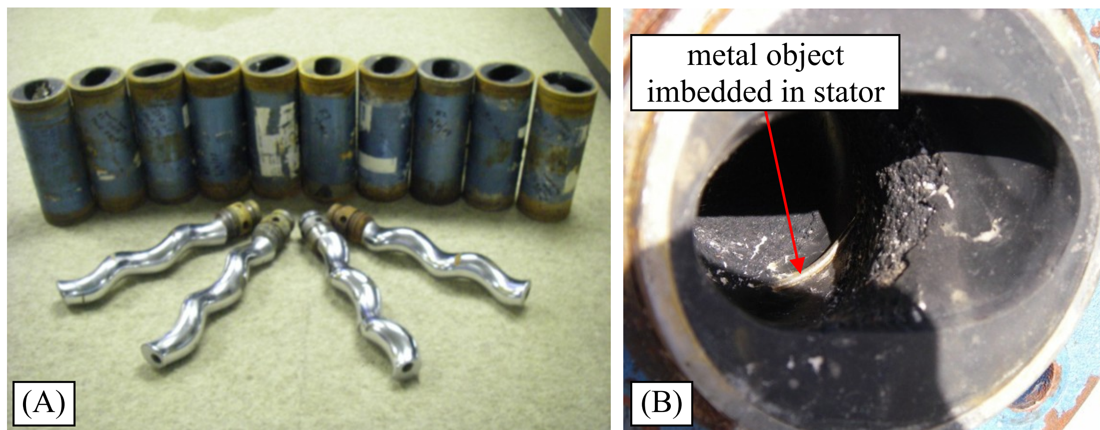


Figure 2-20. (A) Ten stators and four rotors that were damaged between September 2006 and October 2008. (B) Inside a damaged stator.

Figure 2-21. Picture of biomass leaking out around pump packing.

Figure 2-22. Two 3-inch gate values that were removed from fermentor recycle loops because biomass jammed valve in position shown.

Solid handling

Because the pilot fermentation was not automated, operation was very labor intensive. No equipment was used to assist with solids handling (i.e., feedstock addition, transfer of solids between fermentors). Solids were collected and weighed in 5-gallon buckets and manually transferred between fermentors. The movement of large amounts of solids by handmade operation physically demanding and slowed the rate solid transfer could be executed; thus, transfers took more time. For the purposes of this pilot fermentation, manual solid transfer was employed. It was manageable but labor intensive. If scale and/or throughput rate is to be significantly increased, automated solid handling is necessary.

2.3. Tools

2.3.1. Thermometers and temperature control

To monitor the fermentation, the biomass temperature inside each fermentor was

measured at least once per day. During the pilot operations described in this section, different thermometers were used (Figure 2-23). The following subsections describe advantages and disadvantages of each thermometer with respect to the pilot operation. The last subsection describes the temperature control scheme.

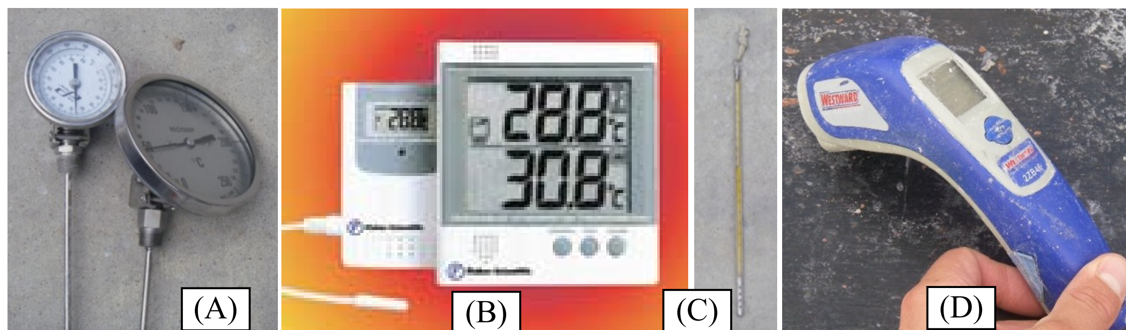


Figure 2-23. (A) Bimetallic thermometers, (B) digital thermometer with wire probe, (C) glass thermometer in metal sheath, (D) infrared surface temperature gun.

Bimetal

Before renovation, each fermentor was equipped with a threaded PVC port near the top-center of the fermentor through which a bimetal thermometer (Figure 2-23A) was installed. During renovation, these thermometers were removed. To test the accuracy and linearity, each thermometer was use to measure the temperature of an ice-water bath (0˚C) and boiling water (100˚C). All the thermometers were inaccurate (>5˚C). The error resulted from the thermometers getting bent by a mixing instrument. These thermometers could be recalibrated by adjusting the position of the graduation plate; however, this was only a one-point calibration and all thermometers had lost the one-to-one linearity.

Digital thermometers with probe

To replace the bimetal thermometers, digital probe thermometers (Figure 2-23B;

Fisher Cat. # 15-077-51 & 15-077-51) were purchased. The thermometers were installed in a sealed case attached to the port-hole lid of each fermentor so that the wire probe hung down into the biomass when the lid was closed. Initially, these thermometers worked and allowed an operator to read the temperature while the fermentors were closed. Within a few months, the hot outdoor temperature began to effect the LCD display and eventually the temperature was unreadable. It was never confirmed, but operators believed that the coating on the wire probe was incompatible with the fermentation broth.

Glass thermometers with metal sheath

Glass thermometers (Figure 2-23C) housed in a protective metal sheath were used as back-up and/or primary thermometers. Each thermometer was tied to a string. To take a fermentor temperature, the loop at the end of the string was hooked outside the fermentor and the thermometer was stuck into the biomass. After 5–10 minutes, the thermometer was removed and the temperature read. The advantage of these thermometers was they were reliable and had no material compatibility issues. The disadvantage was that the temperature changed as soon as the thermometer was removed from the biomass; thus, temperature had to be read quickly or the reading procedure repeated. Sometimes biomass would stick to the thermometer or the thermometer would roll in its sheath and obstruct the reading. Additionally, the glass thermometers, despite being in a metal sheath, would break if accidently dropped.

Infrared gun thermometer

To replace the digital-probe and glass thermometers, a handheld infrared (IR) gun thermometer was purchased (Figure 2-23D). This thermometer was very convenient because it took a quick reading and did not require touching the biomass. Because the IR gun only measured the surface temperature, a temperature reading was taken immediately after the surface biomass had been disturbed. The primary disadvantage was the thermometer would malfunction if it got wet. Compared to the other three thermometers, this was the favorite among operators.

Temperature control

Temperature control is necessary to stabilize fermentation rate and achieve steady state. Although fermentation is an exothermic reaction, the pilot fermentors could cool faster than heat was generated if the outside temperature was sufficiently low. To maintain temperature, each fermentor was equipped with a closed-loop hot water heating system (Figures 2-9 and 2-10). To control the fermentation temperature, the hot water temperature was manual adjusted based on the observed temperature and temperature trend over a moderate period of time (e.g., two weeks).

To investigate if temperature control could be improved, a PID controller was installed to control the temperature of the water in the hot water loop prior to re-entering the water heater. This control scheme was chosen so that no probes had to be placed in the fermentor where they would likely be damaged by the mixing tool. No improvement in temperature control was observed. Improving temperature control was a low priority because the current scheme could maintain temperature within 5°F and day-to-day operations were more demanding; thus, it was not fully understood why the controller did not improve temperature control. The following are hypotheses:

- The controller was not properly setup because the controller manual was difficult to understand.
- The water heater controller overrode the PID controller.
- The controller was redundant; thus, no benefit was perceived.

2.3.2. Mixing tool

Recycling the fermenting biomass via the progressive-cavity pump and 3-inch recycle loop provided minimal mixing and was insufficient to entrain material near the tank perimeter. Manual mixing was required to agitate and homogenize (i.e., equally distribute solid and liquid) the fermentor contents. Prior to renovation, manual mixing was accomplished by an operator standing on a rolling ladder using a square-head shovel. The square-head shovel was not long enough to reach the farthest point from the port-hole. To maximize reach with the square-head shovel, an operator had to stick his

arm into the fermentor, typically below the level of the biomass. Not only was this scenario unpleasant, it did not achieve good mixing. Further, the square-head shovel had sharp corners that gouged (but not ripped open) the tank wall; thus, its use was an unnecessary risk to tank integrity. A better mixing tool was needed.

The catwalk replaced the need for the rolling ladders and provided a superior vantage point to access the fermentors. To allow operators to mix while standing on the catwalk, a 10-foot long aluminum mixing tool was fabricated (Figure 2-24). The distance from the port hole to the far-side bottom corner of the fermentor was approximately 8 feet. The 10-foot mixing tool provided sufficient length that several feet of the tool shaft would protrude from the fermentor when mixing the most distant corner. To allow the operator to twist the mixing tool, a T-shaped handle was incorporated in the design.

In the original design, the ribbed head was detachable so that other head designs could be tested. The ribbed head proved to be sufficient. With use, the connection between the head and the shaft wore and became loose. The pinned connection was removed and the head was welded to the shaft. To allow several fermentors to be mixed simultaneously, two additional mixing tools were fabricated.

Figure 2-24. Ten-foot long aluminum mixing tool with ribbed head.

2.3.3. Mixing ring

From the catwalk using the mixing tool, an operator had great leverage and articulation to manually mix the fermentors. Although mixing was improved, the risk of damaging the plastic tank by prying against the lip of the port hole was increased. To prevent the tank from being damaged, a removable "mixing ring" was created that was mounted over the port hole. Before an operator would use the mixing tool, the mixing ring was placed over the port hole, then the mixing tool inserted into the tank. To turn over thick biomass, the operator could pull and lever the mixing tool against the inside edge of the mixing ring without compromising the tanks. Figure 2-25 shows pictures of the mixing ring in use.

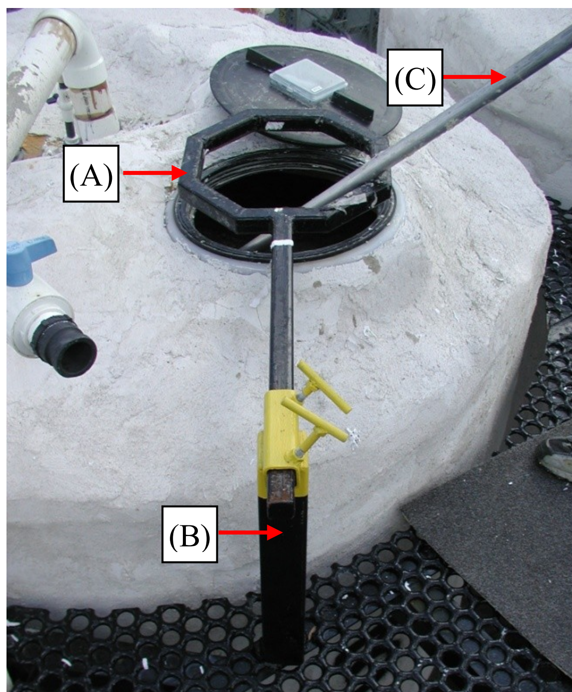


Figure 2-25. Picture of mixing ring in use with objects identified. (A) Detachable mixing ring used to protect plastic fermentor, (B) mixing ring stand, (C) mixing tool.

2.3.4. Volume dipstick

Before the catwalk was constructed and the protective mixing rings installed, volume measurement of fermentor contents was limited to a visual guess. To perform a mass balance, a more accurate volume measurement was needed. A graduated volume "dipstick" was an obvious and inexpensive solution.

Deformities around the port hole of some fermentors would have required a unique volume dipstick for each fermentor. However, the top face of the mixing ring provided a uniform plane (i.e., same for all four fermentors) from which the headspace could be measured; thus, allowing the same dipstick to be used for any fermentor.

When the fermentors were evacuated to remove the internal percolation system (Section 2.1.1), the fermentor internal dimensions were measured (Figure 2-26). From the internal dimensions, the dipsticks shown in Figure 2-27 were created. The stainless

steel dipstick was created because the tape markings on the PVC dipstick were not as durable.

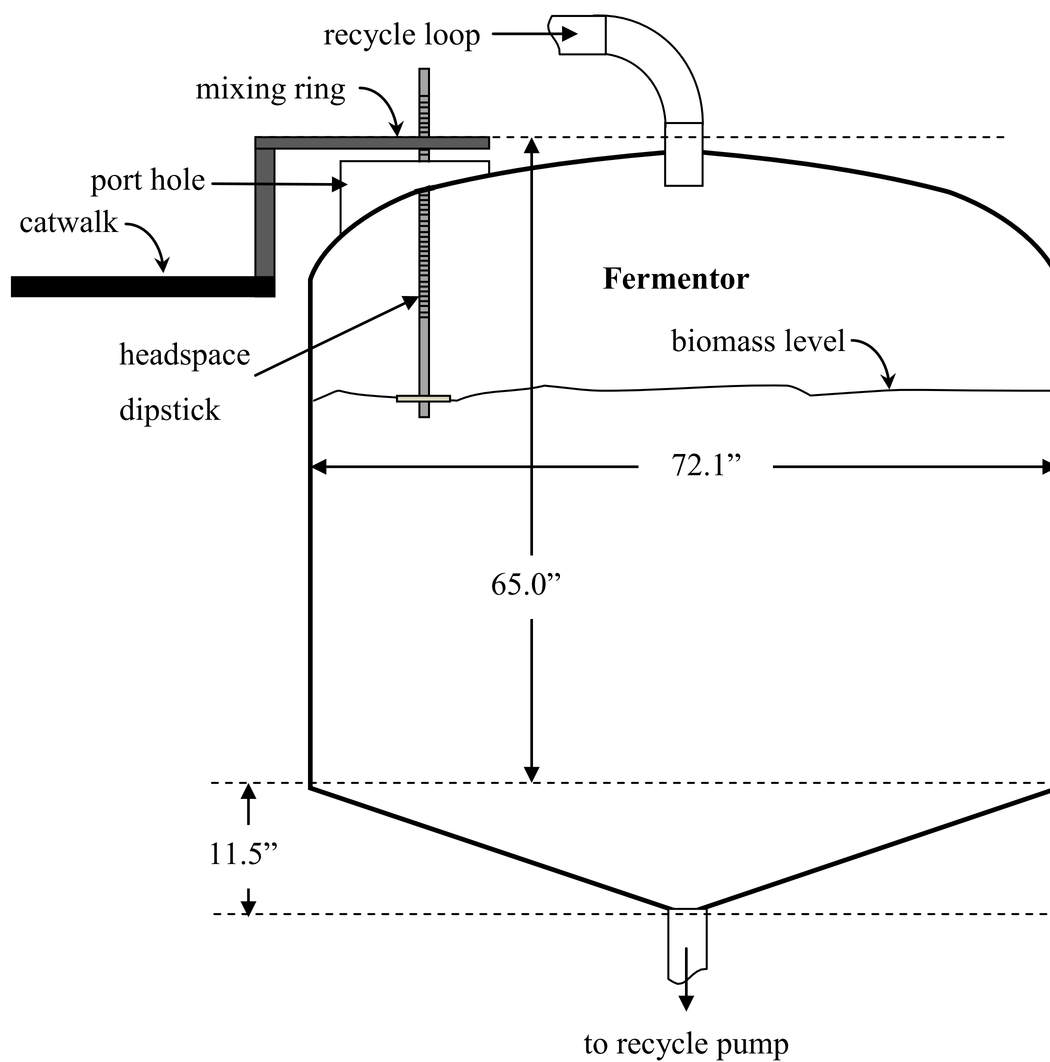


Figure 2-26. Internal dimensions of pilot plant fermentor.

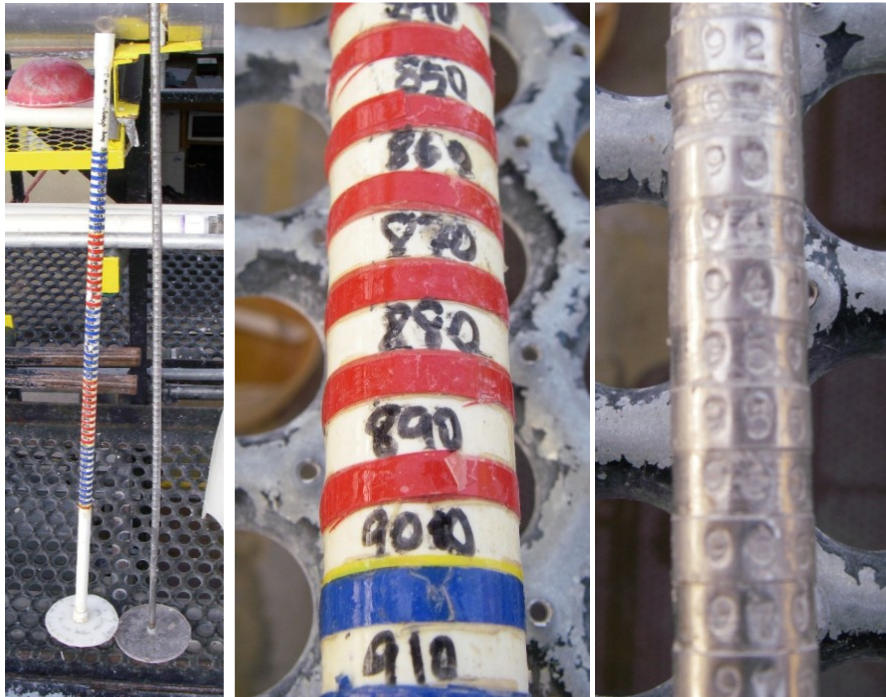


Figure 2-27. Volume measuring sticks with 5-gallon graduations.

2.3.5. Leveling tool

Because the biomass had a high solids content (0.10–0.20 g dry solids/g bulk), there was not excess liquid, which would have allowed the solids to settle below the surface of the liquid. As a result, the top surface of the biomass was not always level. With respect to volume measurement, the variable topography added uncertainty. For example, biomass could accumulate on the far or near side of the port hole, which would cause the volume to be understated or overstated, respectively. The volume discrepancy could be as much as 50 gallons. To mitigate this error, a T-shape leveling tool was constructed from PVC pipe. Prior to volume measurement, an operator would scrape the biomass to be as level as possible. The leveling tool is shown in Figure 2-28.

Figure 2-28. Picture of T-shaped leveling tool made from PVC piping.

2.4. Preservation of product liquor

To provide a supply of unclarified product fermentation liquor to downstream pilot research operations, the product liquor produced from the pilot fermentation described in this dissertation was kept and stored in large (800–1500 gallon) plastic tanks. The storage vessels (Figure 2-29) had a 16-inch portal with a screw-top lid. Each vessel was equipped with a 2-inch quick-connect port with in-line ball valve near the bottom on the tank through which liquor was added. The tanks did not have temperature control and were stored outside (i.e., subject to weather).

Once the first storage vessel was filled, the acid concentration was monitored. Before the stored liquid was sampled, a submersible pump was lowered through the top portal. The pump was allowed to recirculate the tanks contents for 15–45 minutes. Before the pump was turned off, a liquid sample was collected for analysis. Typically, methane inhibitor (800 mL of 20 g iodoform/L 200-proof ethanol) was added each time the tank was mixed. Unfortunately, records were not kept on which days methane inhibitor was, and was not, added.

Figure 2-29. Plastic tanks used to storage fermentation product liquor. The red arrow indicates the storage tank with the product liquor that was monitor.

From Figure 2-30, some acid degradation with time was observed. In the short-term (~2 months), the acid concentration was stable around 25 g/L. After about two months, degradation at a rate of ~1 g/(L·month) was observed until the acid concentration stabilized around 20 g/L. This degradation period coincided with the warmest time of year. After one year, 20% loss in acid concentration (i.e., total acid) was observed.

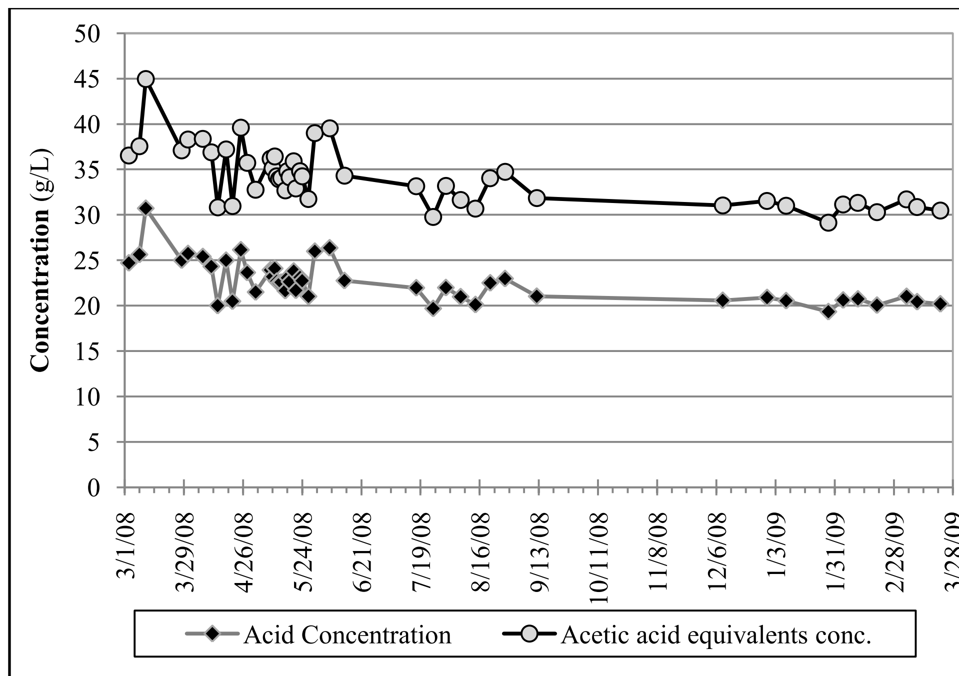


Figure 2-30. Acid and acetic acid equivalents concentration for pilot plant fermentation product liquor in outside storage vessel.

Although the data in Figure 2-30 clearly show that product liquor is relatively stable post fermentation, the observed degradation pattern may not be absolute. There are many factors that can influence the stability and "shelf life" of fermentation liquor:

- storage length (time)
- concentration/population of methanogens
- pH
- concentration/addition of methane inhibitor
- temperature
- air-exposure
- substrate/solid concentration

Ideally, storage of product liquor will be avoided or minimized. However, understanding the stability of product liquor in storage and best management practices

for storing product liquor could be useful in emergency situations or logistic optimization. For example, a downstream unit operation may have a mechanical failure. Backup storage may be necessary to divert product liquor generated upstream. Or, if fermentations are distributed (i.e., located near the biomass source), product liquor may need to be stored for a period of time (e.g., weeks or months) until enough liquor is generated to justify shipment to the next facility.

This simple study provides an order-of-magnitude estimate of the stability of fermentation product liquor in outdoor storage containers. To better understand the influence of the above-listed factors, more rigorous studies are required.

2.5. Conclusions

The following conclusions are made:

- Cleanliness, organization, and functionality are critical to safe and effective pilot plant operation.
- Manual sorting of paper is cost prohibitive.
- The solid-liquid composition and density of each stream must be known and accounted for a mass balance to accurately control the fermentor volume and solid concentration.
- When pumping high solid content slurry, use a valve with minimal inside surface geometry that can cause the valve to foul because of solid accumulation.
- A screwpress dewaters fibrous biomass very well, but is insufficient when particle size is dramatically decreased by increased digestion.
- Product liquor is relatively stable in storage.

3. SOLIDS CONCENTRATION MEASUREMENT

The purpose of this section is to explain (1) the steady-state strategy used at the pilot plant and need for solid concentration measurement, (2) the different mathematical expressions of solid concentration, which govern the different analytical methods (Section 3.2), (3) the characteristics of a solid concentration method (Section 3.3), (4) the different methods that were evaluated during pilot operation (Section 3.4), and (5) conclusions and recommendations based on solid concentration measurement research.

3.1. Steady-state strategy

The goal of the pilot fermentation was to achieve steady state so that performance could be measured and compared with the CPDM model (Section 4). Steady state is achieved when all time derivates equal zero (i.e., there is no change with respect to time). To achieve steady state on the pilot scale, the strategy was to maintain all controllable variables constant with time until dependent variables attained steady state. The critical variables to control were the solid-liquid composition in each fermentor, the fermentor volumes, the solid feed rate, and product liquid flowrate.

To control the solid-liquid composition and volume in each fermentor of a multi-staged countercurrent fermentation train, material balances must be made around each fermentor. Because there are two components to be controlled (i.e., solid and liquid), a system of two equations with two unknowns defines the material balance. Two of the following three balances can be used: total material, dry solids, and liquid. A total material balance (volume) and a dry solid material balance (mass) were used to control the pilot fermentation.

Countercurrent multi-stage fermentations (Figure 1-4) have four major peripheral streams (i.e., inlet and outlet streams): solid feed (i.e., feedstock), solid exit (i.e., waste solids), liquid feed (i.e., water feed), and liquid exit (i.e., product liquid). Other inlets may include nutrient addition to individual fermentors (Section 6 and 7). Of the four major peripheral streams, the flowrates of two are unknown (i.e., dependent variable). The flowrate of all other peripheral streams must be defined (i.e., independent variables).

In the laboratory, because of the high level of control (e.g., entire fermentor contents can be weighed, separated, and staged), the solid feed to Fermentor 1 and liquid feed to Fermentor 4 are typically the independent variables. On a large scale, selection of independent variables that are on opposite ends of the fermentation train is logistically impractical. Defining the solid feed rate (i.e., feedstock feed rate) and the liquid exit rate (i.e., product liquid) is the most logical choice because these periphery streams are (1) on the same end of the fermentation train and (2) include the solid feed and product liquid, which are the streams of greatest operational interest. Controlling these streams at steady state is only a matter of accurate measurement and maintaining a constant transfer frequency. Based on this steady-state strategy, the solid and liquid stream rates between fermentors will eventually come to steady state if the solid-liquid composition and volume are maintained at steady state.

Because the total amount of material in the fermentor (Section 2.3.4) and transfer liquid were most easily measured by volume, the total material balance was on a volume basis; it was not possible to weigh the entire fermentor nor liquid-measuring (volume) tanks. The volume of the transfer solids was calculated from the measured mass and density. The density of the transfer solids was periodically measured by water displacement or estimated using Equation 4-8, which is discussed in Section 3.2.5. To apply a dry solid mass balance, the total mass of dry solids in the fermentor has to be calculated. Because the total amount of material in the fermentor could only be measured by volume, the dry solid concentration (dry lb/bulk gallon) needed to be measured to convert volume to mass dry solids.

Accurate and precise measurement of the dry solid concentration was the greatest technical challenge of the pilot fermentation operation. Conceptually, solid concentration is a simple measurement. In practice, the solid concentration measurement is complicated by uncertainty of representative sampling, which is influence by many parameters (e.g., collection point, mixing, settling). The complexity and permutation of methods outlined in this section were required to improve collection and measurement of a representative sample.

3.2. Solid concentration mathematical relationships

The following subsections outline the mathematical relationships used to calculate the dry solid concentration. Each evaluated solid concentration method was based on one of these relationships.

3.2.1. Density

Throughout the following subsections the density of various materials is discussed. A given substance can have different densities depending on the volume basis. For example, the density of wood chips can be reported three ways: (1) "bulk density" is the mass of the wood chips divided by the pile volume, (2) the "particle density" is the mass of a single wood chip divided by its volume, and (3) the "material density" is the mass of the wood chip divided by the volume of a wood chip that has been compressed to exclude air. As another example, the density of salt can be expressed two ways: (1) the "bulk density" is the mass of the salt divided by the volume of a pile and (2) the "material density" is the mass of a single crystal divided by the crystal volume. To avoid confusion, in this section, unless otherwise stated, "density" is the mass of the respective material divided by the volume of air that material displaces, which is consistent with the material density described above. The abbreviation "SLM" refers to any solid-liquid mixture (SLM) of interest. In the context of this discussion, the SLM is the slurry contained within the fermentors; however, these equations could be applied to filter solids and filtrate, which are also an SLM and contain the same basic components: water, volatile solids, and ash.

3.2.2. Direct measurement

The dry solids concentration is defined by Equation 3-1. Methods based on Equation 3-1 are the simplest and most straightforward. First, the volume of a sample is measured (denominator). Then, to determine the total dry solids, the entire sample is dried. The dry solid concentration is calculated by dividing the total dry mass by the total sample volume.

$$\text{dry solids concentration} \equiv \frac{\text{dry solids (lb)}}{\text{SLM volume (gal)}} \quad (3\text{-}1)$$

3.2.3. Separation-based measurement

The requirement to dry the entire sample prohibits Equation 3-1 from being applied to samples >500 mL because of limited oven capacity and excessive drying time. To increase the sample size, solid-liquid separation devices can be used. These methods assume that the separated solid cake and liquid have uniform moisture content. The volume of a large sample (e.g., 25 gallons) is measured. The sample is then processed through the solid-liquid separation device. The total mass and volume of the cake and liquid are measured, respectively. Subsamples are taken to measure the moisture content of each. The density of the liquid may be measured or if necessary assumed equal to that of water (8.33 lb/gal). Given these data, the numerator of Equation 3-1 is expanded and the solid concentration is calculated according to Equation 3-2.

$$\text{dry solid concentration} = \frac{(\text{cake})(1 - M_{\text{cake}}) + (\text{vol. liq.})(\rho_{\text{liq}})(1 - M_{\text{liq}})}{\text{SLM volume (gal)}} \quad (3\text{-}2)$$

where,

cake = mass of separation solids (lb)

M_{cake} = moisture content of separation solid cake (g moisture/g as-is cake)

vol. liq. = volume of separation liquid (gal)

ρ_{liq} = density of separation liquid (lb/gal)

M_{liq} = moisture content of separation liquid (g moisture/g as-is liquid)

3.2.4. Density and moisture content measurement

If the solid-liquid separation device does not produce separated solids and liquid with uniform and consistent moisture contents, methods based on Equation 3-2 are not reliable. To decouple the size of the volume measurement and the size of the sample

used to measure the moisture content, Equation 3-1 can be expanded as shown in Equation 3-3. Equations 3-4, 3-5, and 3-6 define SLM density, moisture content, and solid content, respectively.

$$\text{dry solids concentration} \equiv \frac{\text{dry solids (lb)}}{\text{total (lb)}} \times \frac{\text{total (lb)}}{\text{SLM volume (gal)}} \tag{3-3}$$

$$\text{bulk density, } \rho_{SLM} \equiv \frac{\text{total sample mass}}{\text{SLM volume}} \tag{3-4}$$

$$\text{moisture content, } M_{SLM} \equiv \frac{\text{mass moisture}}{\text{mass total}} \tag{3-5}$$

$$\text{solid content, } 1\!-\!M_{SLM} \equiv \frac{\text{mass dry solid}}{\text{mass total}} \tag{3-6}$$

Substituting Equation 3-4 and 3-6 into Equation 3-3, dry solids concentration is expressed as shown in Equation 3-7.

$$\text{dry solids concentration } = \rho_{SLM}(1 - M_{SLM}) \tag{3-7}$$

Methods based on Equation 3-7 use a large sample (e.g., 5 gallons) to measure the SLM density ρ_{bulk}, then a representative subsample (e.g., 100 g) is collected to measure the solid content of the SLM (i.e., $1\!-\!M_{SLM}$).

3.2.5. Function of moisture content only

Generally, as the sample size increases so does accuracy. Therefore, it is best to take the largest sample possible; however, equipment, logistics, and available labor limit the sample size. To remove volume measurement as an input parameter, Equation 3-8 expresses bulk density as a function of moisture content, water density ρ_{water}, and the density of the dry solids $\rho_{dry\ solids}$.

$$\text{SLM density, } \rho_{\text{SLM}} = \frac{1}{\dfrac{M_{SLM}}{\rho_{water}} + \dfrac{(1 - M_{SLM})}{\rho_{dry\ solids}}} \tag{3-8}$$

Substituting Equations 3-8 into Equation 3-1, solid concentration becomes a sole function of moisture content (Equation 3-9).

$$\text{dry solid concentration } = \frac{(1 - M_{SLM})}{\dfrac{M_{SLM}}{\rho_{water}} + \dfrac{(1 - M_{SLM})}{\rho_{dry\ solids}}} \tag{3-9}$$

If the density of water and dry solids are known, then the dry solid concentration can be calculated without measuring volume. Methods based on Equation 3-9 may forego collection of large samples in lieu of increased number of samples, which provide greater statistical information and presumably a more reliable average.

The density of water is constant (8.33 lb/gal). However, the density of dry solids is not constant, but is a function of its composition (ash and volatile solids). Typically, the dry solids density of biomass is 12–15 dry lb/gal. For a given substrate system (e.g., paper and manure), the dry solids density of the biomass can be measured at different stages of digestion (i.e., conversion) and an average density used, or an approximate density may be assumed (e.g., 13.5 lb/gal). If an approximate dry solids density is used, the following points should be noted:

- For the fermentor slurry, the SLM density is biased toward the density of water because the fermentation is mostly water (>80%), which reduces the effect of errors associated with the dry solid density.
- If steady-state operation is the primary objective, when measuring the dry solid concentration, precision is more valuable than accuracy. A dry solid concentration method that is reproducible but inaccurate provides greater consistency than the converse. Even if the dry solid density used is not 100%

accurate, great precision can be attained using Equation 3–9 assuming the moisture content measurement is satisfactory.

- For a given substrate system, the dry solid density does not fluctuate greatly with respect to time and extent of digestion.

3.2.6. Function of moisture and ash contents

As stated above, the dry solid density is a function of its composition – primarily ash and volatile solids. To estimate the density of dry solids, Equation 3-10 relates the dry solid density as a function of ash content I_{SLM}, ash density ρ_{ash}, and volatile solid density ρ_{VS}. Equation 3-11 defines ash content.

$$\text{dry solid density, } \rho_{\text{dry solid}} = \frac{1}{\dfrac{I_{SLM}}{\rho_{ash}} + \dfrac{(1 - I_{SLM})}{\rho_{VS}}} \tag{3-10}$$

$$\text{ash content, } I_{\text{SLM}} = \frac{\text{mass ash}}{\text{mass dry solids}} \tag{3-11}$$

Substituting Equation 3-10 into Equation 3-9, dry solid concentration is expressed as a function of moisture content M_{SLM} and ash content $I_{\text{dry solid}}$ (Equation 3-12).

$$\text{dry solid concentration } = \frac{(1 - M_{\text{SLM}})}{\dfrac{M_{\text{SLM}}}{\rho_{\text{water}}} + (1 - M_{\text{SLM}})\left(\dfrac{I_{\text{dry solid}}}{\rho_{\text{ash}}} + \dfrac{(1 - I_{\text{dry solid}})}{\rho_{\text{VS}}}\right)} \tag{3-12}$$

For a dry solid sample containing volatile solids and ash, the density of the volatile solid cannot be measured explicitly. To determine if the ash density and volatile solid density were constant for a given substrate system, the density of the dry solid, density of the ash, and ash content could be measured and the volatile solid density calculated. To make these measurements, dry solids samples were obtained from the pilot fermentation of paper and chicken manure. Samples were taken at different extents

of digestion (i.e., different ash contents). A portion of the dry sample was taken to measure the ash content.

To measure the density of ash and dry solids, a liquid displacement procedure was created using a small volumetric flask (50 or 100 mL) and an analytical balance (±0.001 g). This technique failed to measure the density of dry solid samples because paper fibers captured air bubbles thereby distorting the volume measurement. To accurately measure the density of the dry solids, a gas pycnometer is required. Because ash particles do not readily capture air bubbles, water displacement techniques can be used to measure ash density.

In lieu of experimental data, the nominal densities of the volatile solids and ash can be estimated from the density of known volatile solid and ash compounds (Table 3-1). Table 3-1 shows that most volatile solid compounds have densities of 9–13 lb/gal. According to Sierra et al. (2008), the volatile solid fraction of lignocellulose is predominately cellulose (38–50%) with nominally equal parts hemicellulose (23–32%) and lignin (15–30%); thus, the density of the volatile solids will be biased towards the density of cellulose and hemicellulose (xylose), which are very similar. Based on the above composition and the data in Table 3-1, the nominal density of the biomass volatile solids in the fermentation is 12 lb/gal (1.45 g/mL).

The density of ash is a function of the furnace temperature and mineral profile. The furnace temperature influences the ash composition by further decomposing compounds (e.g., above 825˚C, $CaCO_3$ (23 lb/gal) decomposes to CaO (27.9 lb/gal) and CO_2) and can volatilize some metal compounds (Misra et al., 1993). In the literature (Domke et al., 2004; Forrest et al., 2010; Fu and Holtzapple, 2009; Ross and Holtzapple, 2001) and this dissertation, ash content is typically measured using a furnace temperature of 550–600˚C. Although the mineral composition of biomass can vary widely, the predominate ash compounds formed at 550–600˚C are SiO_2, $CaCO_3$, MgO, and potash (K_2O, K_2CO_3, KCl, and other potassium compounds), which when combined typically account for >80% of the total ash mass (Jenkins et al., 1998; Misra et al., 1993). The density of these compounds ranges from 16–30 lb/gal (Table 3-1). For the

paper and chicken manure mixture used in the pilot fermentation, the ash density was 19–20 lb/gal. Other reports indicate biomass ash can have densities 16–20 lb/gal. If the ash density is unknown, a nominal ash density of 20 lb/gal (or any value between 19 and 21 lb/gal) is a reasonable estimate. On a wet basis, because the ash content is small, the final solid concentration is not highly sensitive to the ash density. Although, a measured value will not dramatically improve accuracy, it is nonetheless preferred. For example, with a moisture content, ash content, and VS density of 0.8 g moisture/g total, 0.2 g ash/g dry, and 12 lb/gal, respectively, a ash density of 19 versus 21 lb/gal only changed the final solid concentration (via Equation 3-12) by 0.003 lb/gal (0.17%).

3.2.7. Function of SLM density only

In Section 3.2.5, Equation 3-8 was inserted into Equation 3-7 to express the dry solids concentration as a function of moisture content only. The resulting method is independent of volume measurement, but does require a drying step (i.e., time delay) to determine the moisture content. To eliminate the time delay required for moisture content measurement, Equation 3-8 may be solved for M_{SLM} (Equation 3-12) then inserted into Equation 3-7 so that dry solid concentration is a function of the solid-liquid mixture density ρ_{SLM} (Equation 3-13).

$$M_{SLM} = \frac{\rho_{water}(\rho_{dry\ solids} - \rho_{SLM})}{\rho_{SLM}(\rho_{dry\ solids} - \rho_{water})} \tag{3-12}$$

$$\text{dry solid concentration} = \frac{\rho_{dry\ solids}(\rho_{SLM} - \rho_{water})}{\rho_{dry\ solids} - \rho_{water}} \tag{3-13}$$

Assuming the density of dry solids is known (Equation 3-10), the dry solid concentration may be calculated immediately after the density of the solid-liquid mixture ρ_{SLM} is measured.

Table 3-1. Density of known volatile solids found in biomass and major ash compounds formed during combustion at 550–600˚C.

Volatile solid compounds	g/mL	lb/gal
Cells[1]	1.10	9.2
Protein[2]	1.2–1.4	10.2–11.9
Lignin[3]	1.3–1.4	10.8–11.7
Starch[4]	1.50	12.5
Cellulose[4]	1.50	12.5
Xylose[4]	1.53	12.7
Glucose[4]	1.54	12.8
Range	1.1–1.54	9–13
Nominal ρ_{VS}	**1.45**	**12**
Ash compounds	g/mL	lb/gal
Potassium chloride[4]	1.98	16.5
Potassium hydroxide[4]	2.04	17.0
Sodium oxide[4]	2.27	18.9
Potassium carbonate[4]	2.29	19.1
Potassium oxide[4]	2.34	19.6
Phosphorus pentoxide[4]	2.39	19.9
Sodium carbonate anhydrous[4]	2.54	21.16
Silicon oxide[4]	2.63	22.0
Calcium carbonate[4]	2.8	23.3
Magnesium oxide[4]	3.58	29.8
Range	2.0–4.1	16–30
Nominal ρ_{ASH}	**2.52**	**21**
Ash Mixtures	**g/mL**	**lb/gal**
Potash[4]	2.0–2.4	16.5–19.6
Wood ash[5]	2.13	17.8
Paper & chicken manure ash	2.3–2.4	19–20
Range	2.0–2.4	16–20
Nominal ρ_{ASH}	**2.28**	**19**

1 – (Bratbak and Dundas, 1984); 2 – (Fischer et al., 2004); 3 – (Holtzapple, 2003); 4 – (Wikipedia_contributors, 2010); 5 – (Abdullahi, 2006)

Although this method requires volume measurement, it has two clear advantages. (1) No time delay to measure the moisture content thereby allowing real-time data collection for material balance purposes. (2) Density measurement of liquid-saturated solid-liquid mixtures is procedurally very simple and fast. To illustrate this point, a five-gallon bucket of known volume (e.g., 5.37 gallons) was over-filled with material (Figure 3-1). Using a straight piece of PVC pipe, the top was scraped level. Using a hanging scale, an identical bucket was tared and then the filled bucket was weighed. The density was calculated from the measured mass and known bucket volume. Because density measurement of liquid-saturated SLM is simple and fast, multiple measurements may be easily made to increase measurement confidence and provide statistical information.

In a commercial plant, Equation 3-13 maybe used to automate a continuous fermentation. A mass and volume flow meter could be installed on fermentor piping. The ratio of the mass and volume flowrates would be the real-time density of the material in a pipe. The data could then be fed to a computer, converted to moisture content or solid concentration, and used in conjunction with a material balance to control the solid-liquid composition and volume. To crosscheck accuracy and/or update constants (i.e., ash content, ash density, dry solid density; Equation 3-10), samples would be collected periodically (e.g., daily).

3.3. Method characteristics

Solid concentration measurements are a complex procedures consisting of many components, all of which can influence the measurement. Each method has two macro steps: (1) sample collection and (2) sample analysis. Each macro step has discrete procedures. The following subsections discuss each component and presents relevant points when defining a solid concentration method. All solid concentration methods were evaluated on fermentations of 80% office paper and 20% wet chicken manure, on a dry basis. It is conceivable that other substrates might behave differently than is described.

Figure 3-1. Five-gallon bucket being used to measure the density of fermentation slurry at pilot plant.

3.3.1. Sample collection

The greatest challenge of any solid concentration method is to obtain a representative sample of the solid-liquid in the entire fermentor. Sample collection has two steps: mixing and sample removal. Within these steps, the sampling device, collection point(s), and sampling tier must be considered. All technique variations were investigated in an attempt to collect the most representative sample.

Mixing

As a policy, when operators were not present, the sludge pumps that recycled

fermentation slurry to the top center of the fermentors were not run. Thus, prior to mixing, the fermentor contents were stagnant for 15–30 hours depending on the operators' schedules. This was enough time for the biomass to stratify. Depending on the solid concentration and level of digestion, different stratifications were observed. High solid concentrations would reduce stratification; lower solid concentration would exacerbate stratification. Less digested biomass (i.e., Fermentor 1) would trap fermentation gas bubbles and float. More digested biomass (i.e., Fermentor 3) would not trap bubbles and would sink. Stratification creates a solid concentration profile within the fermentor thereby biasing samples collected from the top and bottom ports. Further explanation of stratification is noted by Moody (2006).

The time that elapses after the fermentor is mixed affects sample collection and the solid concentration measurement. Three sampling options were considered: before, after, and throughout mixing. The development of the "straw" (details next subsection) allowed quasi-cross-sectional samples to be collected. With this capability, before-mixing sampling was reasonable. If the solid concentration was too low, the slurry would be too runny for a full straw sample to be taken after mixing. However, a before-mixing sample was possible because the settled solids would plug the straw thereby retaining the sample while the straw was removed from the fermentor. Before-mixing sampling should be avoided unless a reliable cross-sectional sample can be obtained.

Technically, the fermentation is a heterogeneous mixture of solids and liquid. However, in the context of this section, "homogeneous" and its variations are used to describe a mixture that has an even distribution of solids and liquid throughout (i.e., no spatial variations in solid concentration).

Sample collection after mixing is logical because the fermentor contents approximate homogeneity. From experience, 10–25 minutes of manual mixing with the mixing tool (Section 2.3.2) in conjunction with sludge pump recycling was sufficient to homogenize. Homogeneity was a subjective determination based on the following:

- Visual solid-liquid composition. Was the apparent composition and consistency of the material returned by the recycle loop the same as the rest

of the material on the exposed surface of the fermentor?

- How the slurry felt when mixing with the mixing tool. Could clumps be felt? Was the relative difficulty of manual mixing the same throughout the fermentor? Qualitatively, were there spatial variations in viscosity?

The third option was to sample intermittingly while mixing. The testing procedure collected samples 10, 15, and 20 minutes after mixing began. This protocol allowed for multiple samples to be collected that differed with respect to slurry that was nearest the sampling points.

For most of the pilot operation, the solid concentration was sufficiently high (i.e., slurry was very thick) that settling would not occur immediately after manual mixing was paused to sample the fermentor via the top port hole. However, occasionally during Trails 1–5 and during Trial 6, the solid concentration in Fermentors 2 and 3 was sufficiently low that solids would settle immediately when manual mixing was stopped; thus, collecting representative samples more difficult. The solid concentration where immediate settling occurred was not absolute, but was influenced by the particle density and size, which are related to the extent of digestion. There are insufficient data to meaningfully correlate the settling rate with dry solid concentration, solid density, and particle size. If it is desired to operate a low-solid-concentration fermentation, mixing equipment must be able to entrain the solids in the liquid so that a homogenous sample can be collected.

Collection point

For the pilot fermentation, samples could be collected from the lower port of the recycle loop and/or through the top port. Stratification, settling, and mixing can bias the solid concentration of samples collected from either point. The points from which samples were collected were well mixed; thus, if slurry in a hard-to-reach part of the fermentor was not mixed and had a different solid concentration, the solid concentration would be biased. Theoretically, if homogeneity is achieved, all sampling points should have the same solid concentration. Comparing values from each sampling point

provides feedback about mixing quality.

If samples are collected from more than one point, the weighting of the corresponding data can greatly impact the final solid concentration value. For example, assume two samples are taken – one from top and bottom of a fermentor. If these data are averaged, then it is assumed that each sample represents half of the fermentor. However, because of the way the fermentor stratifies or settles, the bottom sample may represent the bottom 60% of the fermentor; thus, each sample does not represent the fermentation equally. When designing a fermentor, incorporating numerous (i.e., as many as possible) sampling ports both vertically and laterally will increase the resolution with which the solid concentration can be measured and thereby improves control.

Sampling device

When manually collecting a sample from the top port, the sampling device can influence the solid concentration. The collection devices considered were a short-handled scoop, a long-handled scoop, and a "straw." A scoop is an obvious tool. The set-point volume maintained the top surface of the slurry within arm's reach; thus, slurry near the port hole could be collected with a small bucket or scoop (i.e., short-handled scoop). Compared to a short-handled scoop, a long-handled scoop was preferred because it allowed slurry to be collected from points further from the top port (i.e., beyond arm's length) thereby increasing the effective sampling points.

The sampling "straw" retrieves a slurry sample in the same way a drinking straw removes a sample of liquid by capping the end with a finger. To create a sampling straw, a 1.5-inch brass ball valve was attached to a 4–5 foot piece of 2-inch PVC pipe. Figure 3-2 illustrates how the straw collects an approximate core sample. The motivation for creating the straw was that a core sample would overcome spatial solid concentration variations caused by stratification. Additionally, the straws length allowed the hard-to-reach areas of the fermentor to be sampled. This device was used in five of eight methods investigated (Methods 4-1B, 4-1C, 4-2B, 4-7A, and 4-7B). Typically, 5–10 straw samples were combined and homogenized in a large vat. Using two-tier sampling, subsamples were taken to measure the solids concentration. With experience,

comparison of solids concentration measurements using the straw verse other methods suggested that the straw may have a bias towards collecting more solids than liquid.

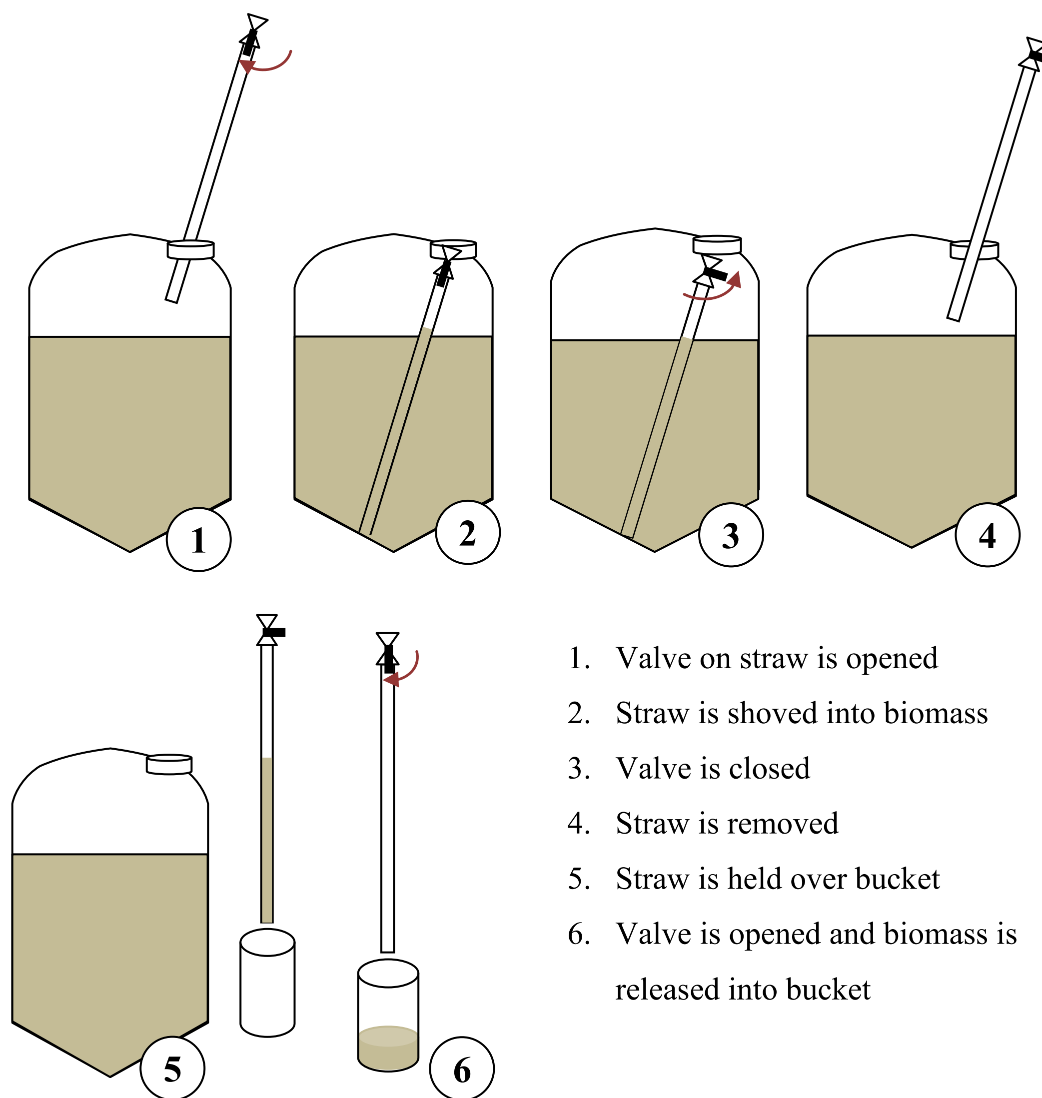


Figure 3-2. Stepwise illustration of how the straw sampling tool was used to collect a quasi-core sample.

Sampling tier

The first tier of sampling involves taking a direct sample, regardless of how it is collected. If spatial variations in the solid-liquid composition exist, a small sample size will be less accurate. To mitigate spatial variations, several direct samples can be collected and combined in a medium-sized vat (10–20 gallons). Because several different direct samples were combined, presumably the collection is more representative of the fermentation than any single direct sample. The combined volume is too large to be collectively analyzed (i.e., dried), but is small enough to be easily homogenized via manual mixing. Once the combined volume was manually homogenized, a subsample (i.e., a second-tier sample) was collected for analysis.

3.3.2. Analytical technique

Once a sample has been collected, the second macro step is analysis. Important factors are the sample size, number of replicates, use of solid-liquid separation, drying technique, and volume measurement. Each analytical technique is governed by an equation discussed in Section 3.2.

For the pilot plant operations, the analytical technique had the following practical constraints:

- Solid concentration is an essential measurement that must be simple and time efficient; a tedious time-consuming procedure was not practical with respect to available financial and labor resources.
- Analysis had to be done on-site. Transport of samples to and from the laboratory – which was several miles away – unnecessarily increased labor and turnaround time.
- Drying equipment was limited to one microwave oven and one convection oven. Funds were not available to purchase additional drying capacity.
- Tools and instruments used to be robust. The pilot plant was a dirty and rugged work environment; fragile and sensitive equipment could easily be damaged.

Variations in sample analysis were developed to improve accuracy and precision while working within these constraints.

Sample size and/or replicates

In theory, as sample size increases, measurement error deceases; thus, to improve accuracy and precision, larger samples are preferred. The true solid concentration is total dry solid mass divided by the total volume. However, to make this measurement is impractical. Increasing the number of samples (i.e., replicates) provides statistical information (i.e., error) and a presumably more accurate measurement. In general, given finite resources, sample size and number of replicates are inversely related. Several analytical method permutations tested were an effort to optimize the tradeoff benefits of size and replicates.

To determine the solid concentration, there are three basic measurements that are input into the equation discussed in Section 3.2: volume, total (wet) mass, and moisture content. The containers, equipment, instruments, and time available set the practical total sample (i.e., (size) × (replicates)) limits for each of these measurements. The following subsections discuss the resources, limits, and issues related to each of the three basic measurements.

Sample volume

For direct methods (Equation 3-1), the total volume and dry mass of a sample are measured explicitly; thus, the sample volume size is limited by the drying capacity (i.e., oven size). For the oven used at the pilot plant, samples had to fit in a 500-mL beaker or smaller; typically, sample volumes were ≤ 400 mL. Relative to the total fermentor volume (800 gallon), 400 mL represents about one ten-thousandth (0.013%) of the total volume. With such a small sample, seemingly small fluctuations can cause great variability in the solid concentration, which then causes variability in the transfer amounts calculated from the material balance.

To increase the sample volume, methods based on Equations 3-2 and 3-7 were developed, which decouple the sample volume from the sample size dried in the oven.

For these methods, 5-gallon buckets (actual volume = 5.37 gallons; ~0.67% of 800-gallon fermentation) were used to measure the sample volume. Because the vast majority of material handling was manual, a 5-gallon bucket was an optimal container; it had the largest volume that could be handled without the filled weight being unmanageably heavy (> 50 lb). To further increase the sample volume or replicates, multiple buckets were used. The primary limitations on volume size and replicates were time and staging-space. To use a 5-gallon bucket to measure volume, the bucket had to be overfilled and then leveled (Figure 3-1), weighed, and data recorded. Although, these steps were quick and easy, iterating the procedure much more than five times per fermentor would have added additional labor without significantly improving accuracy or precision. Because of the order of operations, material had to be staged (i.e, set in piles and groups) until it could be separated or added to the appropriate fermentor. Because the pilot plant had limited space, increasing the sample volume would have further constricted the work space.

For example, on a transfer day, solid concentration samples were taken after mixing. If a solid-liquid separation method was used, the samples would be processed when solid-liquid separation for the respective fermentor was initiated. Solid-liquid separation began with Fermentor 1 and proceeded in order. To maximize use of labor, Fermentors 2 and 3 were mixed and sampled several hours prior to the respective initiation of solid-liquid separation; thus, the samples from those fermentors had to be staged until they could be processed.

The largest volume used for any method was ~27 gallons (five 5-gallon buckets), which represented ~3.3% or 1/30th of the total fermentor volume (i.e., 800 gallons). This volume was sufficiently large that it was considered a representative sample; however, it was too large for replicate measurements. For replicate solid concentration measurement, the volume of a single measurement was limited to one 5-gallon bucket. For separation-based methods (Equation 3-2), the filtrate liquid was measured in 30–50 gallon tanks with 1-gallon increments.

Total (as-is) mass

Because sample volume and total mass are coupled, the limitations with total sample mass measurement are virtually identical to those discussed above. With regard to the measurement of an individual quantity (e.g., one 5-gallon bucket), the scale had a capacity limit of 80 lb.

Moisture content

Of the three basic measurements (volume, total mass, and moisture content), moisture content was the most constrained because of limited drying capacity (i.e., oven space). Limited financial resources prevented purchase of additional ovens. Because the oven capacity was finite, sample size and number of samples that could fit in the oven at one time were inversely related. Although large samples were preferred as discussed previously, drying time increased with sample size. Over the course of pilot operation (i.e., not all changes were made simultaneously), to increase the throughput of moisture content samples, the following procedure changes were made:

1. The oven temperature was increased from 105°C, which is typical, to 130°C. The first solid concentration method tested was a direct method which required 200–400 mL of sample to be dried. At 105°C, these samples required >24 h to dry. Increasing the temperature accelerated the drying process so that the samples dried in <24 h, which was more important than using the "proper" temperature. Additionally, because steady state was the focus of operation, precision was of greater value than accuracy.
2. In lieu of large samples, sample size was decreased to ≤100 g and replicates were increased. Use of two-tier sampling mitigated sampling noise (spatial variation). A sensitivity analysis demonstrated the error contributed by the bench-top scale was insignificant (± 0.002 g moisture/g total).
3. Petri dishes were used, which allowed sample to spread out thereby increasing the exposed surface area and reducing drying time. To increase the number of Perti dishes that could be placed in the oven, metal test tube racks, which had three levels, were used as Petri dish shelves; thus, nine Petri dishes could be

dried in space that three had previously taken.

4. If moisture content analysis was needed immediately, a microwave was used to accelerate the drying process. Over 90% of the moisture could be evaporated in 10 minutes or less. This would at least provide the minimum moisture content. One hazard with using the microwave was if heated for too long, the sample could begin to burn; thus, samples had to be monitored carefully while in the microwave. After preliminary moisture content data was obtained, samples were placed in oven to finish drying.

Solid-liquid separation

To determine the mass of dry solids, the sample can be analyzed as-is or separated. Of the equations outlined in Section 3.2, only one describes separation-based analysis (Section 3.2.3). The motivation for separation-based methods is that by using a filter that produces separation solids and liquid of consistent moisture content, the solid concentration of much larger samples can be measured in a quasi-direct fashion without having to dry the entire sample. Additional benefits include (1) based on recent moisture content data for separation solids and liquid, the solid concentration can be estimated. This provides virtual real-time data that is useful for performing material balances on transfer days. (2) On transfer days, the separated solids and liquid can be applied toward the amounts specified by the material balance; thus, the invested effort serves two purposes.

3.4. Method evaluation

3.4.1. Validation

One challenge with vetting each solid concentration method is that the true solid concentration was never known. To know the true solid concentration, the entire contents would have to be evacuated and dried, which was not practical. To vet a solid concentration technique, one might propose an experiment in which a smaller pilot fermentation (e.g., 55 gallons) of known solid concentration is created. Such an

experiment is not useful because it does not mimic the mixing and sampling problems in the actual fermentation. To vet the accuracy and precision of each method, the following checks were used:

1. Based on experience, did solid concentration measurement match how thick the biomass appeared and felt?
2. How well did the solid concentration agree with other methods?
3. If replicate measurements were made, how precise were the values?
4. Was the precision good enough to match operational trends? For example, while in batch mode, the total dry mass in a fermentor should decrease with time or remain approximately constant. A positive trend in total dry solids implies liquid is converted into solid – an illogical trend.
5. Assuming all other inputs to the material balance were correct, how well did the solid concentration control the fermentation?

Check 1 is qualitative and not very useful unless the apparent discrepancy is very large. Unless multiple methods agree, Check 2 can be confusing because it may not be apparent which is correct. Checks 3 and 4 were the most quantitative; however, discrepancies could also be a reflection of poor mixing rather than poor measurement technique. During Trials 1–3, because the material balance did not fully account for the solid, liquids, and volume of each stream, the solid concentration was not well controlled; thus, applying Check 5 was more of a check on the quality of the material balance than the solid concentration technique. In hindsight, more time and resources should have been devoted to designing and executing a rigorous solid concentration method study.

3.4.2. Methods tested

During pilot operation, eight distinct solid concentration methods were tested. These methods are categorized and referenced by the governing equation. Permutations of each equation method are denoted with a letter. Table 3-2 outlines the details of each

method in terms of the characteristics discussed in Section 3.3. The following subsections review the advantages and disadvantages of each method.

Method 3-1A – direct method

This method was the original method, which was created by the previous pilot fermentation researcher (Moody, 2006). Procedurally, this method is straightforward and simple (Equation 3-1). The disadvantages of this method outweighed its simplicity. The small sample volume made it difficult to obtain representative sample; results were inconsistent and highly sensitive to spatial variations. Because the sample was analyzed in a 500–800 mL beaker, there was not enough oven space for replicates to be dried. Because the samples took more than 24 hours to dry, next-day turnaround was not possible; thus, on the transfer day, less solid concentration data was available from which the measured solid concentration that is entered into the material balance can be determined.

Method 3-1B – direct method w/straw sampling

To reduce sampling error, the sample collection used in Method 3-1A was modified. The straw was used to collect three or four samples, which were collected in a single 5-gallon bucket. The combined sample was manually mixed and then a subsample was collected (i.e., two-tier sampling) and analyzed as specified in Method 3-1A. Method 3-1B has the same basic problems as Method 3-1A. Because a beaker was use to measure volume, the precision was coarse (2–3% error). Unlike water, the slurry sample had a surface topography that added more uncertainty to the volume measurement. Additionally, it was noticed that the biomass trapped many bubbles, which also contributed volume error. During and after Trial 1 when Methods 3-1A and B were investigated, the solid concentration was so high that the fermentation was not saturated with liquid, which may have eliminated bubbles and alleviated volume error. The methods were not revisited to determine if the technique worked better with liquid-saturated slurry.

Table 3-2. Summary of parameters for eight solid concentration methods evaluated.

	Method/Equation	**3-1**			**3-2**		**3-7**		**3-9**
	Permutation	**A**	**B**	**C**	**A**	**B**	**A**	**B**	**A**
Sampling	**Mixing**	after	before & after	after	after	after	after	10, 15, & 20 min of mixing	10, 15, & 20 min of mixing
	Collection point	top	top	top	bottom & 50/50	50/50	top	top & 50/50	50/50
	Sampling device	scoop	straw	straw	pump	straw and pump	straw	straw	scoop and pump
Analytical	**Equation**	3-1	3-1	3-1	3-2	3-2	3-7	3-7	3-9
	Sampling tier	1	2	2	2	2	2	2	2
	Volume measurement	beaker	beaker	displacement	filter tank, 5-gal bucket	5-gal bucket	5-gal bucket	5-gal bucket	n/a
	Vol. sample size	200–400 mL	400 mL*	125 mL	15–20 gal	26.9 gal	5.4 gal	5.4 gal	n/a
	# of replicates	1	1	1	1	1	1	3 or 6	6
	Separation	bulk	bulk	bulk	filter tank	screw press	bulk	bulk	bulk
	Drying sample size	200–400 g	400 g	125 g + water	400–700 g	~100 g (FS) ~500 g (FL)	400–600 g	35–50 g	35–50 g
	Drying dish	beaker	beaker	beaker	beaker	Petri dish (FS) & beaker (FL)	beaker	Petri dish	Petri dish
	Drying	oven	oven	oven	oven	Oven & microwave	oven	Oven & microwave	Oven & microwave
Trial used or tested		1	1*	1*	2 & 3	4–6	2 & 3	3	5 & 6

*indicates method was tested but not used for material balances.

Method 3-1C – direct method with straw sampling and water displacement

To reduce bubbles and volume error, the analytical component was modified. Rather than measure the sample volume in a beaker, a 200-mL graduated cylinder was used to measure the sample volume via water displacement. The method was successful at eliminating bubbles; however, the procedure was so tedious that it would have been time prohibitive and presented challenges training unskilled labor. Method 3-1C was tested but not used for transfers. A rugged less cumbersome method was preferred. Because of its technical merit, future work should revisit water displacement techniques.

Method 3-2A – separation-based method with filter tank

To increase the sample volume and reduce noise, this separation-based method was created. A known volume of slurry (~18 gal) was measured and then poured into the filter tank (Figure 2-14). Vacuum was pulled on the filter tank. Once filtration had ceased, the volume of filtrate and mass of filter cake were measured and samples from each were analyzed for moisture content. Then using Equation 3-2 the solid concentration was calculated. Filtrate density of 8.34 lb/gal was assumed.

The key assumption of this method is there are no spatial variations, with respect to the moisture content, throughout the filtrate and filter cake. With regard to the filtrate, this is a fair assumption because the filtrate liquid can be easily mixed before it is sampled for moisture content. The filter cake had moisture content variations throughout. To mitigate this, multiple aliquots of cake were taken from different portions of the filter cake and combined into one moisture content sample thereby more accurately representing all the filter cake. The data from this method was noisy and inconsistent, but better than Methods 3-1A–C.

Method 3-2B – separation-based method with screw press

Trial 4 was characterized by the switch from the filter tank to the screwpress. Unlike the filter tank, the screwpress produced separation solids with a uniform moisture content thereby reducing the noise observed with Method 3-2A. The sample size was increased to ~27 gallons (i.e., five 5-gallon buckets). Half of the sample was obtained

from the top port and half from the lower port of the recycle loop. The separated solids and liquid could be used for transfer and therefore required minimal additional effort; thus, this method was maintained throughout Trials 4–6. The advantage of this method was that a large sample could be consistently analyzed in a short time (15–30 minutes). Because the screwpress produced separation solids and liquid with a uniform and repeatable moisture content, the solid concentration could reliably be estimated from previous moisture content data in real-time without having to wait for the moisture content samples to dry.

Method 3-7A – density and moisture content method

An evolution in technique occurred when Equation 3-7 was proposed. The method was simple and effective. Using a 5-gallon bucket of known volume and mass, straw samples were collected until the bucket was overfilled. Then, using a straight piece of pipe, the bucket was leveled, weighed (Figure 3-1), and the density calculated. The content of the bucket was emptied into a large vat and mixed well. Then, a 400–600 g subsample was placed in a beaker for moisture content analysis. Because of the beaker size, limited oven space, and long drying time, replicate measurements could not be made.

Method 3-7B – density and moisture content method with Petri dish

To increase the number of samples that can be dried in the oven at one time, Petri dishes were used as described previously. Because replicates could be taken, the sample collection procedure was modified such that one sample was taken from the top and bottom of the fermentor after 10, 15, and 20 minutes of mixing. Six measurements provide a reliable average and error, which could also be used to evaluate the degree of homogeneity achieved (i.e, smaller error = better mixing). The drawback to this method is that the increased amount of data could add confusion when choosing the solid concentration value to enter into the material balance. (For more details on choosing a solid concentration value, see Section 3.4.3.)

Method 3-9A – moisture content only method

Assuming the material densities (i.e., ρ_{VS}, ρ_{ash}, $\rho_{dry\ solids}$) are reasonably accurate, Equation 3-9 allows the solid concentration to be calculated as a sole function of moisture content. In lieu of making a large volume measurement, numerous moisture content measurements can be made using the Petri dishes. In this case, six samples were analyzed; one sample was taken from the top port and bottom port after 10, 15, and 20 minutes of total mixing. This data set helped increase representation of spatial variations and provided statistical information (error). The increased number of measurements was a mixed blessing. If the values were similar, then there was great confidence in the average. If there was large scatter in the data, choosing a value to input in the material balance was challenging.

3.4.3. Choosing a solid concentration value

Throughout the pilot operations, the measurement of solid concentration evolved. The material balance required the measured solid concentration. Making a judgment about the value that was input was not always straightforward (i.e., simply input data average). The multi-dimensional decision process included quantitative and qualitative judgments, which is summarized by the decision tree shown in Figure 3-3.

The first step in the decision tree is a subjective evaluation of whether or not the data are trustworthy. The apparent thickness and pumping behavior are indicators of the solid concentration. Based on experience, if the data do not match the observed properties of the slurry or the error is very large, then the reliability of the data is questionable.

If the average value was believable, then the implications of that value had to be considered. If the solid concentration was too high, then large amounts of separated solids and liquid may be required to satisfy the material balance. Depending on the amounts required and the separation rate, it may not be possible to satisfy a transfer based on a high solid concentration in one workday. When this was the case, the solid concentration was lowered to a value that resulted in transfer amounts that were

achievable. The solid concentration set point would be re-establish over several transfers rather than all at once, which would dampen extreme oscillations if the original value was overstated.

If the average value was believable but too low, the fermentor would have a net gain of solids. If the solid concentration got too high, then it could become constipated (i.e., would not pump because slurry was too thick), which would prohibit operation and may require the addition of fresh water (not ideal) to reduce the solid concentration to regain operation. When the solid concentration was very low relative to the set point, to reduce the potential risk of constipating the fermentor, the solid concentration value input into the material balance could be increased.

If the data were not considered trustworthy, depending on the dataset, trends and outliers could be inspected to improve the value. If these techniques did not help, a best-guess value was chosen. Ideally, the decision process would be completely objective and based on data; however, because of labor and equipment issues and the uncertainties involved with the solid concentration data "gut-feeling" judgments were necessary. As experience was gained, better material balances were achieved (i.e., material balance more fully accounted for the solid mass, liquid mass and total volume of all streams), and greater confidence in solid concentration measurement was gained; thus, the decision process became more objective.

3.4.4. What was the best method tested?

Without having performed a definitive experiment, the recommendation is Methods 3-2B (separation method with screwpress) and 3-9A (moisture content only with Petri dish). Preferably, both methods should be used to create a dataset from which the solid concentration is determined. Method 3-2B can be easily implemented with a minimal increase in operation time. Its use of a large sample and the reproducibility of the screwpress make this method reliable. Method 3-9A is procedurally simple, improves representation of spatial variations, and provides statistical information.

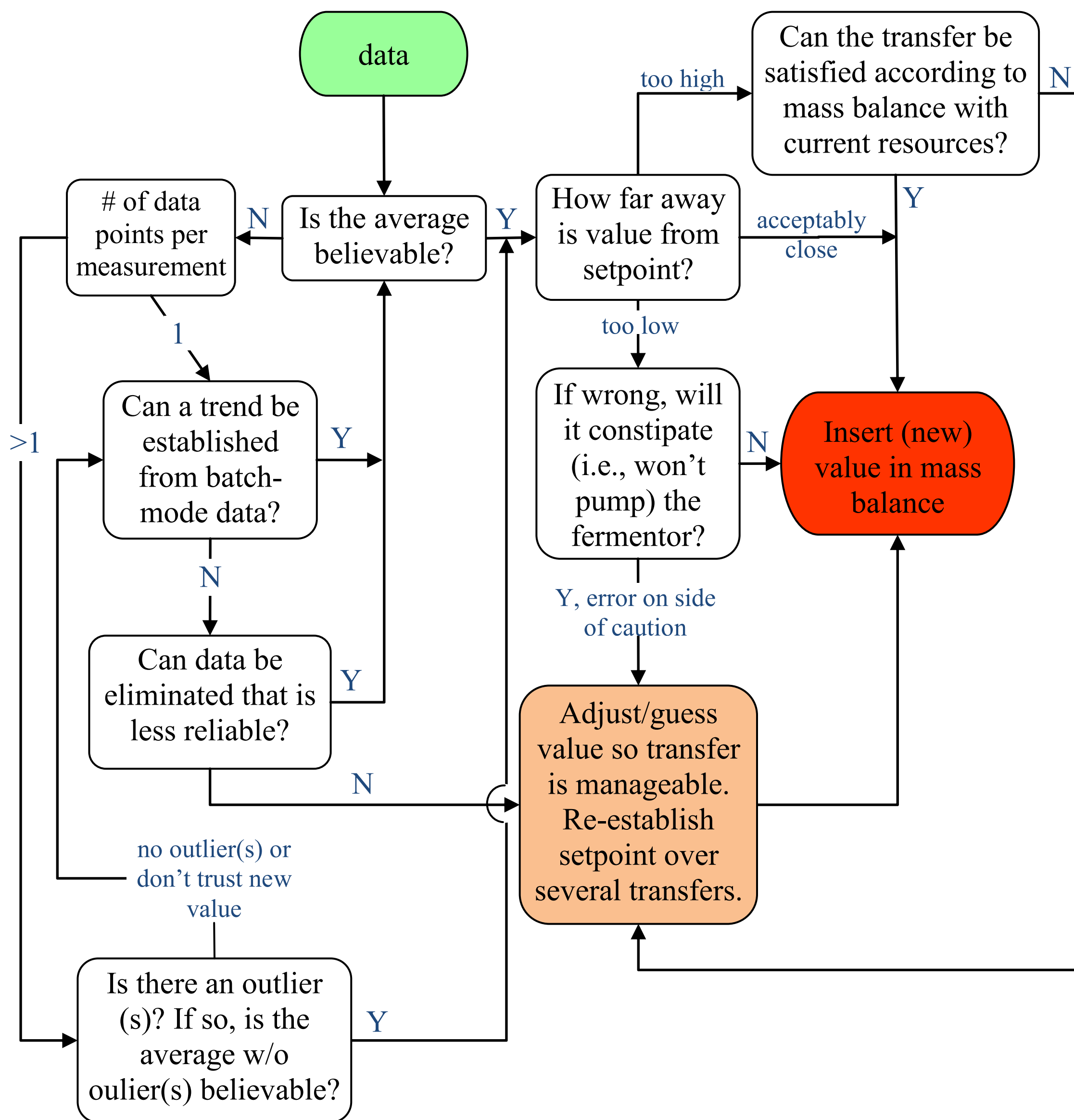


Figure 3-3. Decision tree used to evaluate solid concentration data and determine the value that was entered in material balance calculations. Y and N represent "yes" and "no," respectively.

3.5. Conclusions and recommendations

From the pilot fermentation, much was learned about how to measure and control the solid concentration via material balance. The following are key conclusions and recommendations:

- Collection of a representative sample is the critical challenge when measuring solid concentration.
- Settling rate, stratification, solid concentration, reactor geometry, and mixing schemes all affect the ability to homogenize the fermentor and collect a representative sample.
- Sampling error (spatial variations, sampling bias) was much greater than analytical error. To mitigate sampling error, increase sample size and/or number of samples.
- When designing a submerged fermentation vessel, include vertical and lateral sampling ports.
- In general, after- or throughout-mixing sampling is more reliable than before-mixing sample.
- The straw technique has merit, but further research is needed to counteract its bias towards collecting more solid than liquid.
- If it is desired to operate a low-solid-concentration fermentation, mixing equipment must be able to keep the solids entrained in the liquid so that a homogenous sample can be collected.
- Thorough accounting of the solid, liquid, and volume of each stream is required to accurately control the fermentor volume and solid concentration; update constants (stream moisture contents, material densities, etc.) as frequently as possible.
- The moisture content and bulk density are related by the ash content, and density of water, ash, and volatile solids (Equation 3-8 & 3-12). If ash and volatile solid density data is unavailable, nominal densities of 20 and 12 lb/gal, respectively, can be used.

- Test methods based on Equation 3-13 (solid concentration as a function of density only).
- To measure the density of dry solids, a gas pycnometer should be used.
- Create a rigorous formal experiment to evaluate mixing quality (i.e., degree of homogeneity) and compare and validate different solid concentration methods and techniques.
    - Create fermentation with known initial solid concentration.
    - Dilute fermentation and evaluate solid concentration based on expected change in solid concentration.
    - Remove entire contents and measure solid mass.
- If steady-state operation is the primary objective, when measuring the dry solid concentration, precision is more valuable than accuracy.
- Maintaining a solid concentration low enough that the slurry is saturated with liquid improves volume measurement (both of the fermentor and solid concentration samples) and control.
- If large discrepancies between the measured and set point solid concentration are observed, it is better to adjust the solid concentration in small manageable increments over the course of several transfers rather than all at once, which can result in unmanageable quantities being transferred and excessively long transfer time.

4. COMPARISON OF PILOT FERMENTATION PERFORMANCE WITH CONTINUUM PARTICLE DISTRIBUTION MODELING PREDICTIONS

4.1. Introduction

4.1.1. Brief history

An important goal of research on the MixAlco process^{TM} is to help our industry partner (Terrabon, Inc. of Houston, TX) commercialize the process. Laboratory research on the MixAlco process^{TM} began in 1991. The first patent related to the MixAlco process was issued in 1997 (Holtzapple et al., 1997). In 2001, construction began on the pilot plant discussed in this section. In 2008, Terrabon built a 5-ton-per-day demonstration fermentor (Energy Independence I) to further develop of the MixAlco process^{TM}.

4.1.2. Research objectives

The core objectives of the pilot fermentation were: (1) understand how to achieve steady state (i.e., operate a pilot-scale fermentation), (2) identify and understand operational issues associated with large-scale operation, and (3) determine validity of CPDM to predict performance of large-scale fermentations. Objectives 1 and 2 are addressed in Sections 2 and 3. The purpose of this section is to present data pertaining Objective 3. To focus discussion and avoid redundant descriptions, references will be presented in lieu of repeating details that are more appropriately described in other sections.

Design and optimization of commercial fermentations requires a kinetic model. The continuum particle distribution modeling (CPDM), developed by Loescher (1996), is an empirical method for modeling mixed-acid fermentation. CPDM predicts laboratory fermentation performance reasonably well (more details shown in Section

4.3.1); however, no large-scale fermentations have been compared with CPDM predictions. This is the first research to compare CPDM predictions with a large-scale mixed-acid fermentation.

4.2. Pilot plant

4.2.1. Fermentor design and operation

The design of the pilot fermentation is described in Section 2 and also by Moody (Moody, 2006). The feedstock components and properties are discussed in Section 2.1.2. The steady-state strategy is described in Section 3.1.

4.2.2. Trials

Over the course of this study, six attempts to achieve steady state were made. Each of these attempts is referred to as a "Trial," which are briefly summarizes as follows:

Trials 1 and 2 were short lived; each lasted nominally two weeks. Both trials were terminated because the dry solid concentration measurement technique and the material balance spreadsheet were insufficient to achieve steady state; however, these trials were very useful for procedure development and spreadsheet refinement.

Trial 3 achieved the first steady-state approximation. It used the oilfield-style filter basket (Figure 2-14) for solid-liquid separation. Trial-3 steady state was not perpetuated longer than was necessary because material transfers had become very difficult and it was determined that the filter tank was insufficient to sustain operation. Because the filter tank had low solid-liquid separation efficiency, material transfer required more than 12 hours, which over-burdened the available labor. Further, non-ideal practices had to be used to compensate. For example, during some transfers, the material balance dictated a volume of transfer liquid (L_3) could not be obtained from F3 in a reasonable period of time. To overcome this, fresh water was used to make up the volume deficit.

Trial 4 was a repeat of Trial 3, except a screw press was obtained to replace the filter tank. A second steady state was achieved. Of the six trials, Trials 3 and 4 most closely *approximated* steady state; therefore, only the performance of Trials 3 and 4 can be meaningfully compared to each other and with CPDM predictions. The controllable and normalized operating parameters for Trials 3 and 4 are summarized in Table 4-1.

Trials 5 and 6 were attempts to improve pilot plant performance by nitrogen supplementation (i.e., urea addition). Urea addition increased digestion thereby decreasing particle size. This caused the screw press to operate very slowly such that operation times became impractical. Both Trials 5 and 6 failed because of filtration issues. Because it was not yet understood how to control nitrogen in a countercurrent staged fermentation, it was not practical to continue with further trials. For more details about Trials 1–6, see Section 2.1.

4.2.3. Air exposure

During a material transfer, for a given fermentor (e.g., Fermentor 1), the protocol was to remove exiting amounts first (e.g., S_1 and L_1), then add inlet quantities (e.g., S_0 and L_2). A consequence of this protocol was that transfer solids and liquids had to be staged in the open air, thereby compromising the anaerobic condition. This practice was unavoidable given the manual natural of pilot fermentation operation. Transfer solids were staged in open 5-gallon buckets. Depending on the fermentor, filtration rate, and transfer amounts required, staged material would be exposed to air for 1–5 hours (1.5 hours was typical). To minimize air exposure, lids were purchased and a new protocol was tested. Because the lids further slowed operation and broke easily, the use of lids was abandoned. The transfer liquids were collected and staged in a measuring tank before being transfer to the appropriate fermentor. Although the transfer liquid was more contained and less exposed, its transfer was not completely anaerobic.

Table 4-1. Controllable and normalized operating parameters for pilot-fermentation Trials 3 and 4. Error represents two standard deviations (95% confidence interval).

	parameter	units	Trial 3		Trial 4	
			Set point	Actual	Set point	Actual
Controllable	temperature, F1	˚C	40	41 ± 4	40	38 ± 6
	temperature, F2		40	43 ± 5	40	39 ± 9
	temperature, F3		40	40 ± 5	40	37 ± 7
	transfer frequency	transfers/week	2	1.9 ± 0.1	2	1.82 ± 0.1
	dry solids feed rate, S_0	lb/transfer	125	125 ± 5	125	125 ± 5
	product transfer liquid	gallons/ transfer	50	50 ± 2	50	50 ± 2
	total volume F1	gallons	800	760 ± 24	800	804 ± 12
	total volume F2		800	791 ± 31	800	789 ± 42
	total volume F3		800	801 ± 43	800	798 ± 40
	dry solids conc., F1	lb dry solids/ gallon bulk sample	1.54	1.60 ± 0.1	1.50	1.46 ± 0.1
	dry solids conc., F2		1.93	1.91 ± 0.2	1.83	1.80 ± 0.2
	dry solids conc., F3		2.20	2.14 ± 0.2	1.95	1.91 ± 0.3
Normalized	VSLR	g NAVS/(L_{liq}·d)		1.7 ± 0.1		1.5 ± 0.1
	MRT	d		93 ± 5		95 ± 5
	LRT	d		166 ± 5		182 ± 5
	SRT	d		126 ± 2		126 ± 4
	TLV	L_{liq}		7,513 ± 258		7,719 ± 258
	NAVS conc., F1	g NAVS/L_{liq}		112 ± 32		116 ± 20
	NAVS conc., F2			124 ± 36		164 ± 43
	NAVS conc., F3			129 ± 33		157 ± 43
	moisture content, F1	g liquid/g wet sample		0.814 ± 0.01		0.822 ± 0.01
	moisture content, F2			0.791 ± 0.01		0.796 ± 0.03
	moisture content, F3			0.760 ± 0.02		0.783 ± 0.04
	pH, F1			6.16 ± 0.5		6.11 ± 0.4
	pH, F2			6.08 ± 0.2		6.01 ± 0.4
	pH, F3			5.92 ± 0.5		6.02 ± 0.6

4.3. Continuum particle distribution model (CPDM)

4.3.1. Overview

The mathematics and theory of CPDM have been well described by previous authors (Agbogbo, 2005; Aiello-Mazzarri, 2002; Domke, 1999; Domke et al., 2004; Fu, 2007; Loescher, 1996; Ross, 1998; Thanakoses, 2002); therefore, this section outlines only the key concepts.

A *continuum particle* is a representative sample of discrete particles that has a collective sum of one gram of non-acid volatile solids upon entering the fermentor (Loescher, 1996; Ross, 1998). Ross's continuum particle definition is based on one gram of *volatile solids*; however, this should be one gram of *non-acid volatile solids (NAVS)* because acid products are not reactants. Mathematically, this is just semantics. Experimentally, this change effects the measurement and design of the batch fermentations used to collect kinetic data.

The kinetic parameters are determined from an array of batch fermentations with different initial NAVS concentrations. The acetic acid equivalents (aceq), conversion, selectivity, and initial NAVS concentration data are used to fit the governing rate equation (Equation 4-1).

$$\hat{r} = \frac{e\,(1-x)^f}{1 + g\,(\varphi \cdot A_e)^h} \tag{4-1}$$

where,

$\hat{r}$ = specific rate of reaction

(moles of acetic acid equivalents produced/(g cont. part. · d)

e, f, g, and h = empirical constants

x = conversion of NAVS (g $NAVS_{consumed}$/g $NAVS_{feed}$)

$$\varphi = \frac{\text{moles actual acid *}}{\text{moles aceq}}$$

A_e = acetic acid equivalents (g/L_{liq})

* units discrepancy (see following page for explanation)

Once the specific reaction rate has been determined, mixed-acid fermentations schemes (e.g., countercurrent staged, batch, continuously stirred tank reactor (CSTR)) can be modeled and optimized mathematically using programs such as MatLab.

Since 1996, when CPDM was first developed by Loescher (1996), the method has been refined. Ross (1998) made the following modifications: (1) the definition of a continuum particle was changed from one gram of *solids* to one gram of *volatile solids*, (2) the original six-constant empirical rate equation was simplified to the current four-constant empirical equation (Equation 4-1), (3) to prevent overestimating the inhibitory effects of higher-molecular-weight acids, the coefficient φ was added (Equation 4-1) such that product inhibition is expressed as a function of the actual acid concentration, rather than aceq concentration, and (4) introduced a non-linear regression technique that minimizes the least-squares residuals with the acid concentration data rather than the calculated rate data. Aiello-Mazzarri (2002) showed the accuracy of the CPDM predictions may be improved by accounting for selectivity as a function of volatile solids loading rate (VSLR).

In 1998, when Ross introduced φ (moles actual acid/moles aceq), he expressed acid concentration on a molar basis (i.e., mol acid/L). In 2002, Thankoses, Chan, and Aiello-Mazzarri switched to modeling aceq concentration on a mass basis (i.e., g aceq/L). Thus, the units of φ changed to a mass basis (i.e., g actual acid/g aceq). During the modeling presented in this section, this nuance was overlooked and was not recognized until after simulations and analysis had been completed; unfortunately, mole and mass units were not used consistently. Although use of mixed units is not "proper," mathematically, this discrepancy does not change the modeling results. For example, for the Strict batch fermentations, if mass basis units had been used, φ would be 0.746 g actual acid/g aceq rather than 0.613 moles actual acid/moles aceq. During regression, g would have adjusted to compensate for the value changed of φ (i.e., g would equal 15.04 rather than 14.50) and the specific reaction rate surface would be unaffected. Furthermore, because φ is a constant, φ^h can be incorporated into g (i.e., $g_{new} = g_{old} \cdot \varphi^h$), thereby simplifying Equation 4-1.

Although this simplification was apparent, When Ross introduced φ, he decided to maintain its introduction for the following reasons:

1) there was little CPDM modeling history, it was hypothesized that as experience was gained modeling lignocellulose feedstocks, it would be observed that the empirical constants (particularly g and h) would be nominally constant and only φ would change as the acid product spectrum changed.
2) Because of the above hypothesis, it was preferred to model acid concentration on an aceq basis, and use φ to correct the units so that inhibition is more accurately expressed as a function of actual acid and not acetic acid equivalents.

Now that more CPDM experience has been gained, it has been shown that the empirical constants are not nominally the same across lignocellulose feedstocks (Agbogbo and Holtzapple, 2006; Agbogbo and Holtzapple, 2007; Aiello-Mazzarri et al., 2006; Chan, 2002; Domke et al., 2004; Forrest et al., 2010; Fu, 2007; Ross, 1998). Thus, inclusion of φ is unnecessary until data exist to show that its inclusion adds additional meaningful information. To simplify the specific rate equation, it is recommended that φ be removed.

As of December 2010, CPDM has been used over 100 times to model laboratory-scale countercurrent staged fermentations (Agbogbo, 2005; Aiello-Mazzarri, 2002; Chan, 2002; Domke, 1999; Forrest, 2010; Fu, 2007; Ross, 1998; Thanakoses, 2002). The average absolute error between measurement and CPDM prediction for total acid concentration and conversion is 12.3 ± 13% and 16.9 ± 17% (error represents one standard deviation), respectively. Histograms of acid concentration and conversion error are shown in Figure 4-1. Approximately 48% of the acid concentration predictions and 66% of the conversion predictions were overstated (i.e., prediction greater than measured value). Nominally, 70% of all CPDM predictions are within 20% of the measured acid concentration and conversion. When one considers the complexity of mixed-acid fermentation, the range of substrates, and range of operating parameters used in these studies, this level of accuracy (<20% absolute error) is very good; thus, validating the fundamental *concept* of CPDM.

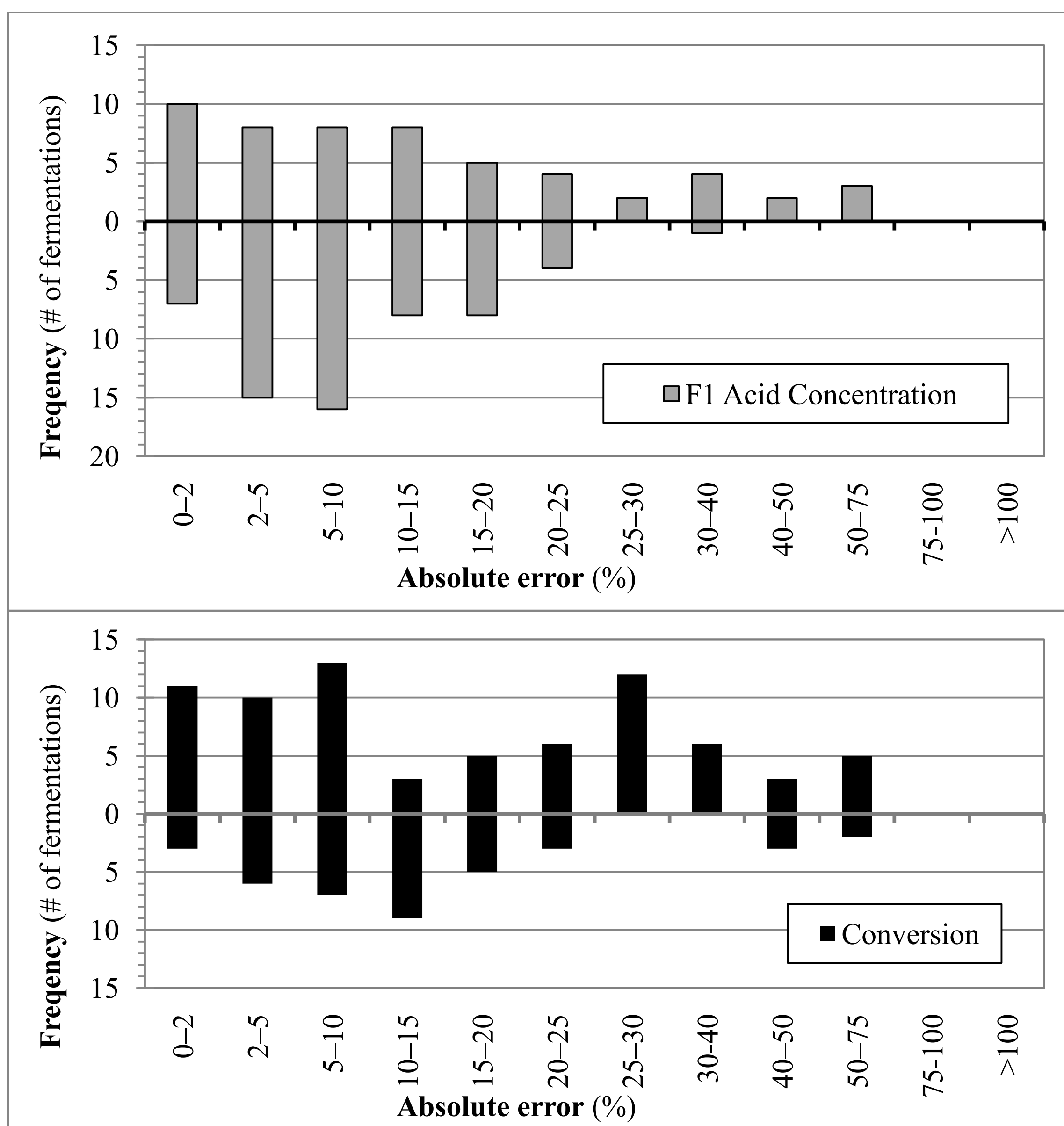


Figure 4-1. Error histograms for predictions of CPDM total acid concentration (g acid/L_{liq}) and conversion (g $VS_{consumed}$/g VS_{feed}) in semi-continuous countercurrent mixed-acid fermentations (Agbogbo, 2005; Aiello-Mazzarri, 2002; Chan, 2002; Domke, 1999; Forrest, 2010; Fu, 2007; Ross, 1998; Thanakoses, 2002). Frequencies above the *x*-axis represent predictions with positive error (overstated). Frequencies below the *x*-axis represent predictions with negative error (understated).

4.3.2. Batch fermentations

For a substrate system (in this case, paper and chicken manure), to provide data from which the empirical constants of the specific reaction rate are regressed, five batch fermentations were performed. The first four had nominal initial non-acid volatile solids (NAVS) concentrations of 20, 40, 70, and 100 g NAVS/L with no additional initial acid. The fifth batch fermentation had a nominal NAVS concentration of 100 g NAVS/L and a nominal additional initial acid concentration of 20 g acid/L (45% calcium acetate, 45% calcium propionate, 10% butyric acid). Each batch fermentation was inoculated with strained fermentation broth from a countercurrent train that used the same substrate system; thus, the mixed culture was adapted.

Strict anaerobic procedures

The standard laboratory protocol for countercurrent and batch fermentations (including CPDM batch fermentations) maintains a strict anaerobic environment. The media (i.e., water; described in Section 6.2.1) is deoxygenated. To prevent air from contacting the fermentation bottles, the transfers are performed under a nitrogen purge. Figure 4-2 shows the acid concentration of each strict CPDM batch.

Relaxed anaerobic procedures

Because CPDM is an empirical model, the kinetic parameters are specific to the chemical and biochemical components present in the batch fermentation (e.g., sugarcane bagasse with sewage sludge, urea, deoxygenated media, calcium carbonate buffer, iodoform methane inhibitor, and no artificial nutrient mix). Although not ideal, as mentioned above (Section 4.2.3), the pilot plant fermentation was exposed to air, which can affect the biochemistry and thereby affect kinetics and performance. If air-exposure is a factor, the CPDM batch fermentations need to incorporate air so that the data more accurately represents the true air-exposed biochemistry.

Every two days, when the built-up pressure was vented and measured, samples were collected, and methane inhibitor was added. In addition, to incorporate air in the CPDM batch fermentations, each fermentation had air bubbled in the fermentation broth

for 15 minutes. This procedure was meant to simulate air exposure during operation of the pilot plant. The laboratory air exposure time (15 min) was estimated to represent a "typical" air exposure in the pilot plant; it is not based on rigorous analysis. The most representative air-exposure procedure would have been to remove the fermentation biomass, separate filter solids and liquids, and allow the material to sit in open air for 90 minutes. Such a procedure would have resulting in significant moisture evaporation and material loss from bottle removal, which would have been difficult to account. The 15-minute bubbling procedure did not require biomass removal and minimized mass lost.

The air exposure observed at the pilot plant inspired a collaborative air-exposure study lead by Kristina Golub (Golub et al., 2011). Her study compared strict and relaxed anaerobic procedures on two four-bottle countercurrent trains. Paper and wet chicken manure were used as feedstock. The relaxed countercurrent train used the 90-minute air-exposure procedure mentioned above. Because the fermentation was semi-continuous, mass losses were less significant than would be observed for a batch fermentation. The relaxed CPDM batch fermentations described in this section were inoculated with strained fermentation broth from Golub's relaxed countercurrent fermentation. Figure 4-3 shows the acid concentration of each strict CPDM batch.

4.3.3. Computer simulation

The computer model and code have been well described by previous authors (Agbogbo, 2005; Aiello-Mazzarri, 2002; Domke, 1999; Domke et al., 2004; Fu, 2007; Loescher, 1996; Ross, 1998; Thanakoses, 2002); therefore, this section outlines only the inputs unique to the pilot plant simulation. Table 4-2 outlines the inputs used to model Trials 3 and 4. The average selectivities of the CPDM batch fermentations were used. The MatLab code was the same described by Forrest (2010) (Appendix E). To convert CPDM aceq concentration predictions to acid concentration, the average aceq ratio of the batch fermentations was used, which is shown in Table 4-2.

The fermentation model is a combination of a reaction model (i.e., Equation 4-1) that describes the fermentation rate and a configuration model that describes the physical

aspects of the fermentation. Figure 4-4 illustrates how general model components are grouped and related within the fermentation model.

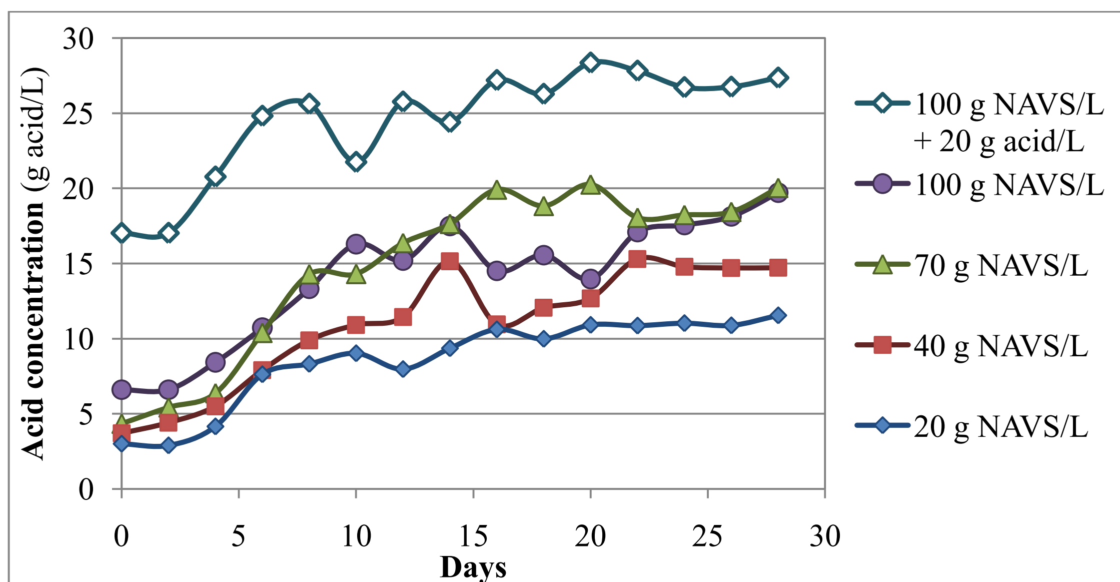


Figure 4-2. Acid concentration for CPDM batch fermentations using strict anaerobic procedures.

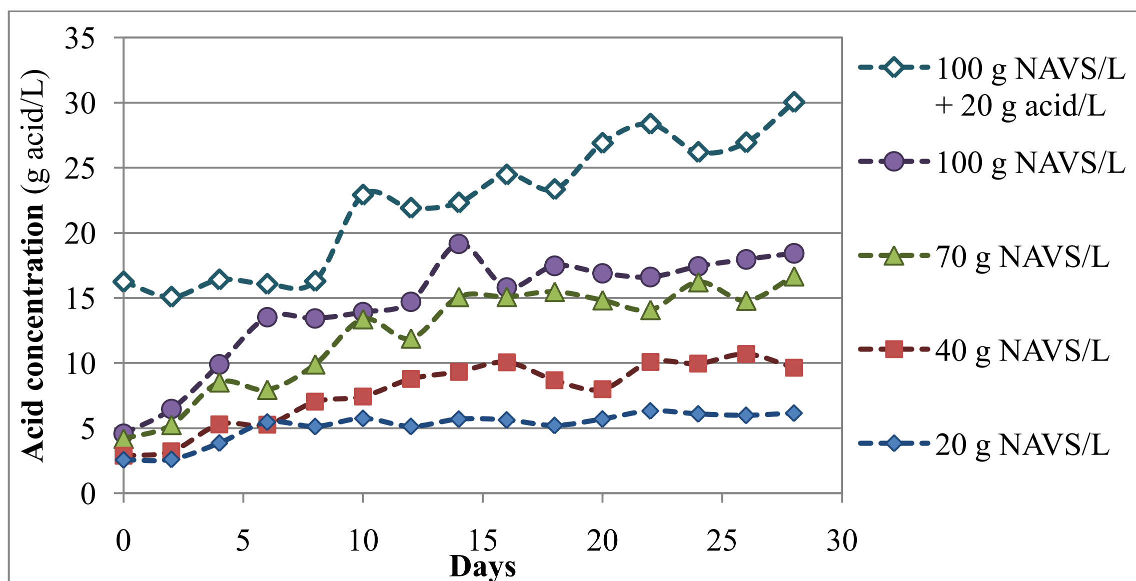


Figure 4-3. Acid concentration for CPDM batch fermentations using relaxed anaerobic procedures.

Table 4-2. Summary of CPDM inputs used to model Trials 3 and 4.

Parameter	Units	Trial 3		Trial 4	
		Strict	Relaxed	Strict	Relaxed
Number of stages	---	3	3	3	3
VSLR	$g/(L_{total}\cdot d)$	1.38	1.38	1.29	1.29
LRT	d	168	168	168	168
Holdup	g liquid/g VS cake	6.49	6.49	6.07	6.07
Moisture content of feed	g moisture/g wet feed	0.349	0.349	0.334	0.334
Selectivity	g aceq/g NAVS consumed	0.67	0.50	0.67	0.50
F1–F3 solid conc.	$g\ VS/L_{total}$	112, 124, 129	112, 124, 129	116, 164, 157	116, 164, 157
F1–F3 total volume	L_{total}	2477, 2541, 2495	2477, 2541, 2495	2662, 2514, 2543	2662, 2514, 2543
φ	mol acid/mol aceq	0.613	0.664	0.613	0.664
e	g aceq/(g NAVS·d)	0.913	1.368	0.913	1.368
f	dimensionless	0.393	2.380	0.393	2.380
g	$L/(g\ aceq)^{1/h}$	14.50	29.50	14.50	29.50
h	dimensionless	0.852	0.590	0.852	0.590
Aceq ratio	g aceq/g acid	1.34	1.31	1.34	1.31

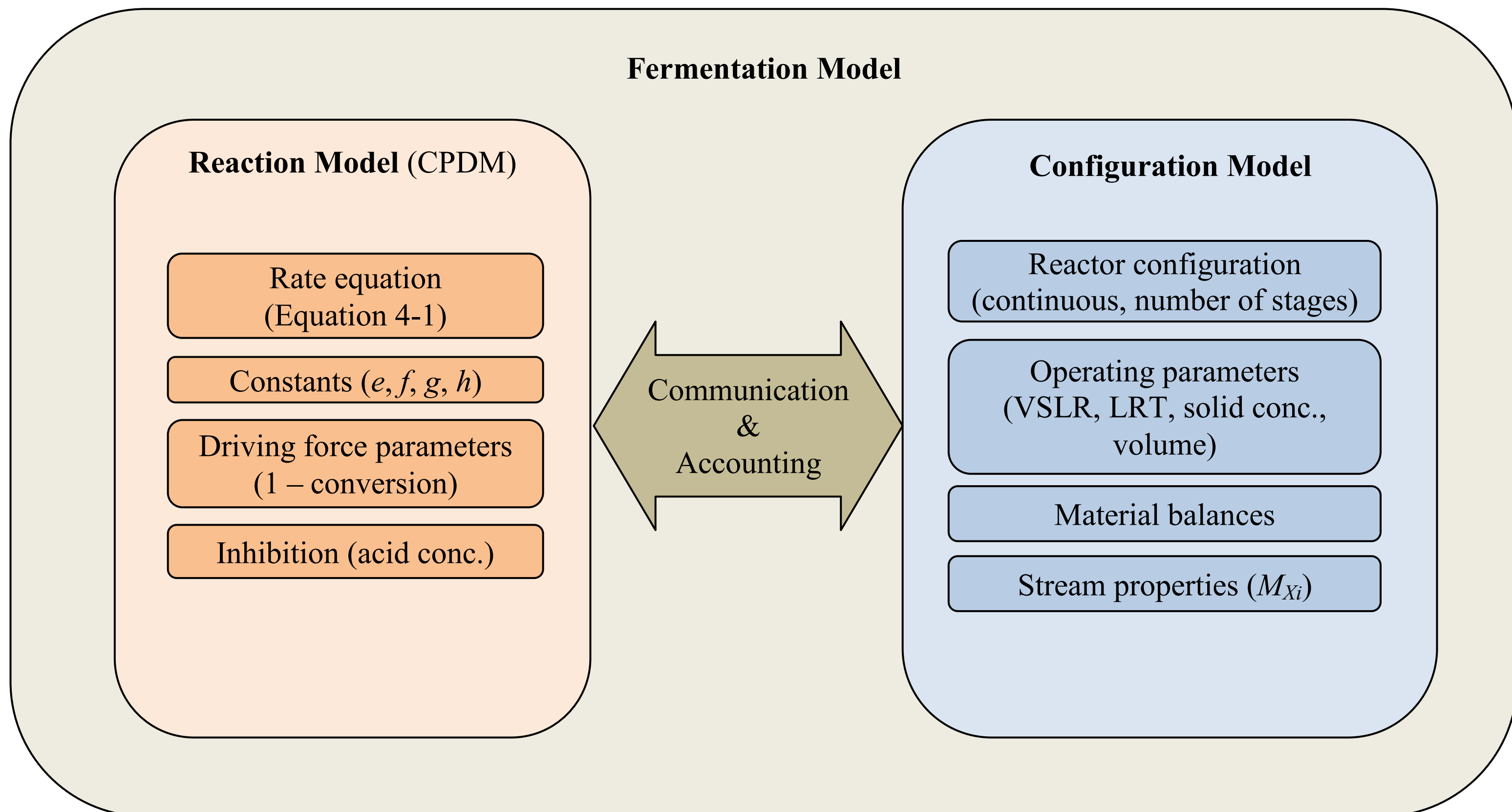


Figure 4-4. Illustration of fermentation model components.

4.4. Analytical methods

4.4.1. Acid concentration and gas composition

The analytical technique for measuring the acid concentration and gas composition are described in Section 6.2.2.

4.4.2. Moisture and ash contents

For pilot plant samples, moisture and ash contents were determined by drying samples using a 130 ºC forced convection oven (>12 h) and a 550 ºC furnace (>3 h), respectively. No lime was added before drying. See Section 3.3.2 for more detail about pilot plant technique.

For CPDM batch fermentation samples, moisture and ash contents were measured in parallel. Before moisture content analysis, 3 g $Ca(OH)_2$/100 g sample was added to ensure all carboxylic acids were retained as carboxylate salts during drying. Samples were dried using a 105 ºC forced convection oven (>12 h). Ash content samples were dried without lime and then place in 550 ºC furnace (>3 h), respectively. The procedure used for the CPDM batch fermentation samples is more refined and had not been determined at the time the pilot fermentation was operated.

4.4.3. Volume (pilot plant only)

Based on the internal dimensions of the conical-bottom fermentors, a dipstick was made to correlate the depth of head space to volume in 10-gallon increments. For more details, see Section 2.3.4.

4.4.4. Slope method (pilot plant only)

To determine the flowrate of a component (acid, ash, NAVS, water, or gas) the moving cumulative sum of that component was plotted with time. The component flowrate (amount/day) was determined from the slope of the line. All pilot plant performance variables were calculated from component flowrates determined by the

Slope method (Smith and Holtzapple, 2010a). For more details on the Slope method, see Section 5.

4.4.5. Definitions of terms

Referring to the labels defined in Figure 4-5, the following terms are used in this paper:

$$\text{NAVS}_{\text{feed}}\ (\text{g}) \equiv \text{sum of NAVS in } S_0\text{, and } L_4 \quad (4\text{-}2)$$

$$\text{NAVS}_{\text{exit}}\ (\text{g}) \equiv \text{sum of NAVS in } S_3 \text{ and } L_1 \quad (4\text{-}3)$$

$$\text{NAVS}_{\text{consumed}}\ (\text{g}) \equiv \text{NAVS}_{\text{feed}} - \text{NAVS}_{\text{exit}} \quad (4\text{-}4)$$

$$A_{\text{feed}}\ (\text{g}) \equiv \text{sum of carboxylic acid in } S_0\text{, and } L_4 \quad (4\text{-}5)$$

$$A_{\text{exit}}\ (\text{g}) \equiv \text{sum of carboxylic acid in } S_3\text{, } L_1\text{, and any liquid samples removed from F1–F3} \quad (4\text{-}6)$$

$$A_{\text{produced}}\ (\text{g}) \equiv A_{\text{exit}} - A_{\text{feed}} \quad (4\text{-}7)$$

$$A_{L_1}(\text{g}) \equiv \text{total carboxylic acid in } L_1 \quad (4\text{-}8)$$

$$\text{conversion} \equiv C \equiv \frac{\text{NAVS}_{\text{consumed}}}{\text{NAVS}_{\text{feed}}} \quad (4\text{-}9)$$

$$\text{yield}_{\text{feed}} \equiv Y_F \equiv \frac{A_{\text{feed}}}{\text{NAVS}_{\text{feed}}} \quad (4\text{-}10)$$

$$\text{yield}_{\text{exit}} \equiv Y_E \equiv \frac{A_{\text{exit}}}{\text{NAVS}_{\text{feed}}} = Y_F + Y_C \quad (4\text{-}11)$$

$$\text{yield}_{\text{culture}} \equiv Y_C \equiv Y_E - Y_F \equiv \frac{A_{\text{produced}}}{\text{NAVS}_{\text{feed}}} = C \cdot E \tag{4-12}$$

$$\text{yield}_{\text{process}} \equiv Y_P \equiv \frac{A_{L_1}}{\text{NAVS}_{\text{feed}}} \tag{4-13}$$

$$\text{selectivity} \equiv \sigma \equiv \frac{Y_C}{C} \tag{4-14}$$

$$\text{productivity (train)} \equiv P \equiv \frac{A_{\text{produced}}}{\text{TLV} \times \text{time}} \tag{4-15}$$

where total liquid volume (TLV) is defined in Equation 4-16.

TLV is the total liquid volume expressed as

$$\text{TLV} = \sum_i \left(\frac{K_{Fi} M_{Fi}}{\rho_w} \left(\frac{1\ \text{L}}{1000\ \text{mL}} \right) + L_{Fi} \right) \tag{4-16}$$

where,

L_4, and S_0, are rates determined by the Slope method (g/d)

K_{Fi} = the average mass of wet solid cake in Fermentor *i* (g),

L_{Fi} = the average volume of free liquid in Fermentor *i* (L).

Liquid retention time or hydraulic residence time are common chemical engineering terms used to quantify the average time for fluid to travel through a reactor or pipe. Generally speaking, longer retention times allow for higher product concentrations.

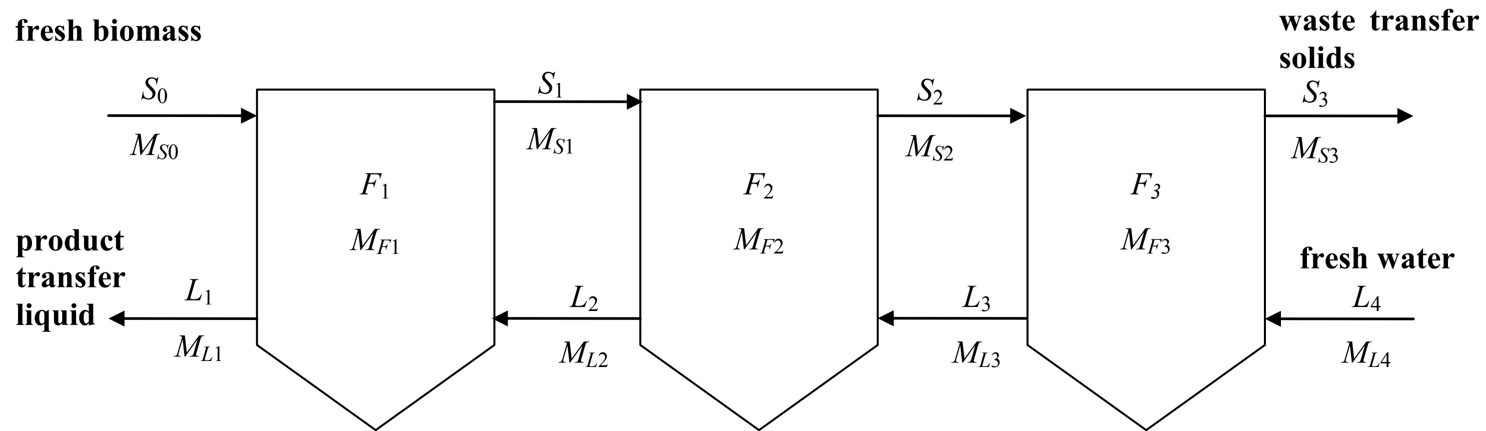


Figure 4-5. Three-stage countercurrent pilot fermentation. S_0 and L_4 are the feed carbohydrate and water stream flow rates (lb/transfer), respectively. S_i, L_i, and F_i are the transfer solids stream flowrate (lb/transfer), transfer liquid stream flowrate (lb/transfer), and total fermentation mass (lb) in Fermentor i, respectively. M_{Xi} is the moisture content in stream or fermentor X_i, respectively, where X represents S, L or F.

With regard to countercurrent mixed-acid fermentations, the traditional definition of LRT (Equation 4-17) is defined as the total liquid volume divided by the product liquid flowrate. From a modeling and design perspective, this definition is very useful; the TLV and LRT can be independent parameters thereby defining the product liquid flowrate, which is required for material balances computed by a computer model and/or designing the size of the fermentation and downstream equipment.

$$\text{LRT} = \frac{\text{TLV}}{Q_{PL}} = \text{liquid retention time (d)} \qquad (4\text{-}17)$$

where Q_{PL} is determined using Equation 4–18 and the Slope method:

$$Q_{PL} = (L_1)\frac{1}{\rho_W}\left(\frac{1\text{ L}}{1000\text{ mL}}\right) = \text{product liquid flowrate (L/d)} \qquad (4\text{-}18)$$

Although LRT is useful for modeling and design, for a countercurrent fermentation, the terminology can be misleading because the definition *does not* accurately measure the average time *liquid* (i.e., water) is retained in the system. As indicated in Figure 4-5, each inlet (S_0 and L_4) and outlet (L_1 and S_3) stream contains moisture; thus, to quantify the average time liquid moisture is retained in the system, the total liquid volume should be divided by the total inlet or total outlet moisture flowrate. To quantify this term, moisture retention time (MRT) is defined (Equation 4-19). For convenience, MRT is defined with respect to the total inlet moisture flowrate for the following reasons:

- The moisture content of the inlet streams is generally more stable than the outlet streams.
- Liquid fed to the last fermentor is typically water (i.e., 100% moisture).
- Evaporative and leakage losses (i.e., unintended outlets "streams") do not have to be accounted.

$$\text{MRT} = \frac{\text{TLV}}{Q_{moisture}} = \text{moisture retention time (d)} \quad (4\text{-}19)$$

where, $Q_{moisture}$ is determined using Equation 4-20 and the Slope method:

$$Q_{moisture} = \left(L_4 M_{L_4} + S_0 M_{S_0}\right) \frac{1}{\rho_W} \left(\frac{1\ \text{L}}{1000\ \text{mL}}\right)$$
$$= \text{total inlet liquid flowrate (L/d)} \quad (4\text{-}20)$$

The ratio of MRT/LRT may be used to quantify the overall liquid separation efficiency, which is useful to quantify the fraction of moisture that enters the system and leaves with the product liquid. As water usage is optimized and filtration performance improves, this ratio would approach unity.

Volatile solids loading rate (VSLR) quantifies the reactant feed rate relative to the total liquid volume and is defined as

$$\text{VSLR} = \frac{\text{NAVS}_{\text{feed}}\ \text{rate}}{\text{TLV}} \quad (4\text{-}21)$$

4.4.6. Determination of NAVS$_{consumed}$

The NAVS$_{\text{consumed}}$ is the difference between the NAVS in the inlet and exit streams. This quantity can be determined by two approaches: (1) inert ash and (2) direct measurement.

Inert-ash approach – Assuming ash is inert, the ash flowrates in and out are equal. Based on this assumption, the difference between the dry material in the inlet and outlet streams results from the change in VS, not a change in ash. The NAVS$_{\text{consumed}}$ rate (g NAVS$_{\text{consumed}}$/d) may be determined by Equation 4-23.

$$\begin{aligned}\text{NAVS}_{\text{consumed}} \text{ rate} &= \text{NAVS}_{\text{feed}} \text{ rate} - \text{NAVS}_{\text{exit}} \text{ rate} \\ &= (\Sigma\text{dry solids}_{\text{in}} - \Sigma\text{ash}_{\text{Iin}} - \Sigma\text{acid}_{\text{in}}) - (\Sigma\text{dry solids}_{\text{out}} - \Sigma\text{ash}_{\text{in}} - \Sigma\text{acid}_{\text{out}}) \\ &= (\Sigma\text{dry solids}_{\text{in}} - \Sigma\text{acid}_{\text{in}}) - (\Sigma\text{dry solids}_{\text{out}} - \Sigma\text{acid}_{\text{out}}) \qquad (4\text{-}23)\end{aligned}$$

where,

$$\text{dry solids in stream } X_i \text{ (g)} = X_i\,(1 - M_{Xi}) \qquad (4\text{-}24)$$

$$\text{acid in stream } X_i \text{ (g)} = A_{X_i} = \frac{X_i\,[A]_{X_i}\,M_{X_i}}{\rho_W}\left(\frac{1\text{ L}}{1000\text{ mL}}\right) \qquad (4\text{-}25)$$

Direct measurement – The NAVS component flowrate in inlet and outlet streams (S_0, L_4, S_3, L_1) are measured directly using Equation 4-22 and the Slope method. The total inlet $\text{NAVS}_{\text{feed}}$ flowrate minus the $\text{NAVS}_{\text{exit}}$ flowrate equals the $\text{NAVS}_{\text{consumed}}$ rate.

$$\text{NAVS}_{X_i} = X_i\left(\left(1 - M_{X_i}\right)\left(1 - I_{X_i}\right) - \frac{[A]_{X_i} M_{X_i}}{\rho_W}\left(\frac{1\text{ L}}{1000\text{ mL}}\right)\right) \qquad (4\text{-}22)$$

where,

X_i = total transferred mass of Stream X_i (g)

M_{X_i}= moisture content of Stream X_i (g moisture/g wet sample)

I_{X_i} = ash content of Stream X_i (g ash/g dry sample)

$[A]_{X_i}$ = total carboxylic acid concentration (g/L_{Liq}) of Stream X_i

ρ_w = density of water (1 g/mL)

The Direct method has been the traditional method for determining the NAVS consumed; however, recent studies (Smith et al., 2011) have indicated that the direct method is less accurate than the inert-ash approach. For Trials 3 and 4, the Direct method had to be used. The pilot fermentation was inoculated with beach soil from Galveston, TX that was composed of marine microorganism, water, partially digested organic matter, and sand, which is inert (i.e., ash). During Trials 3 and 4, as

countercurrent operation progressed, the ash content of the fermentation dramatically decreased as the sand (ash) was purged (Figures 4-6 and 4-7). In this scenario, the inert-ash approach is inappropriate; the direct approach was the only option to estimate conversion, which could be slightly overestimated.

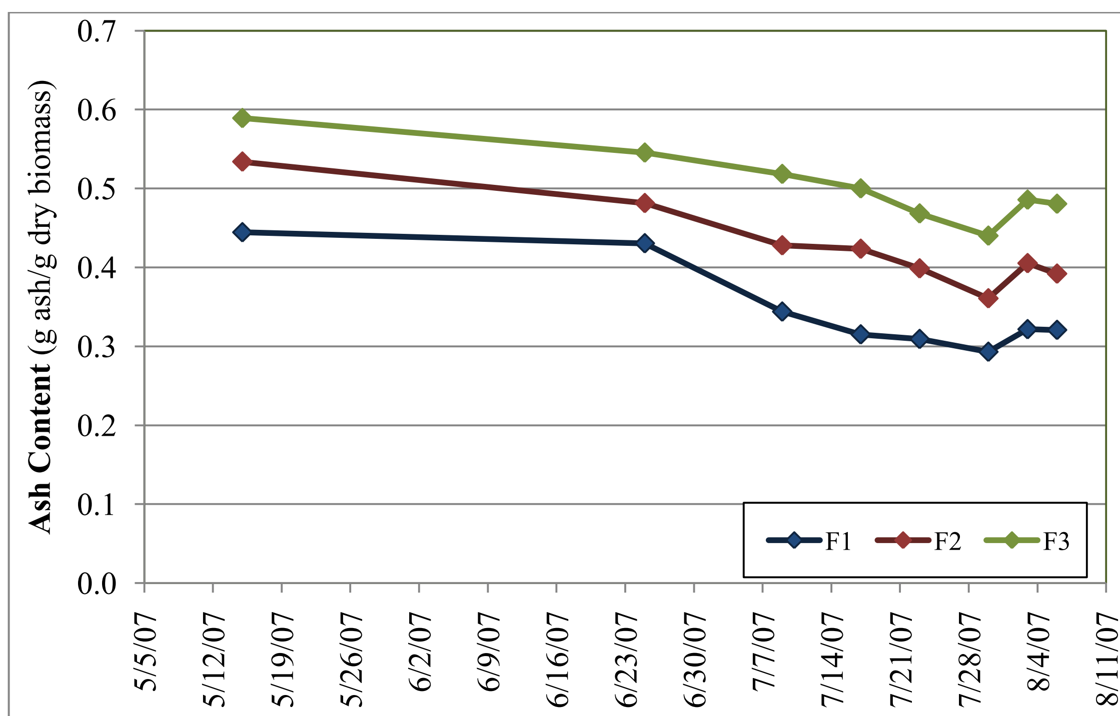


Figure 4-6. Trial-3 ash content profile for Fermentors 1–3 (F1–F3).

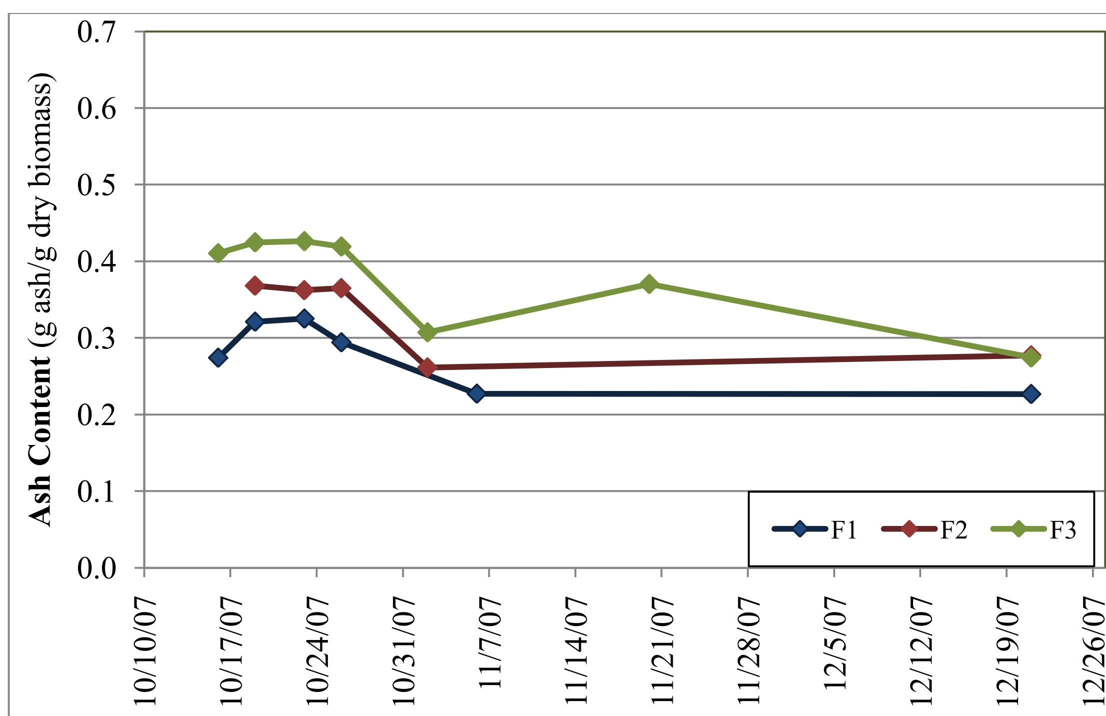


Figure 4-7. Trial 4-ash content profile for Fermentors 1–3 (F1–F3).

4.5. Results and discussion

The following subsections present performance results from Trials 3 and 4, and CPDM predictions of Trials 3 and 4. The performance of Trials 3 and 4 are compared and the agreement of each trial with relaxed and strict CPDM predictions is discussed.

4.5.1. Pilot plant performance

To compare the pilot plant with CPDM predictions, steady state was assumed; the fermentor composition at the beginning and end of the steady state was assumed to be identical. Thus, only the quantities of acid and NAVS in the inlet and outlet streams were used to calculated performance; the change in fermentor composition was ignored. These steady-state performance parameters are summarized in Table 4-3.

Although acid concentration was stable during Trials 3 and 4, true steady state was not achieved. As shown in Figures 4-6 and 4-7, ash content steadily decreased;

thus, the NAVS content of each fermentor increased because dry solids concentration was controlled. The "steady-state" performance parameters shown in Table 4-2 are an *approximation* of true steady state. To achieve a true steady state (i.e., all system variables at steady state – not just acid concentration), the system would have to be operated for multiple LRTs (>3), which would have required 2–3 years of uninterrupted operation. This was prohibitive. Future pilot plant fermentation trials should (1) allocate sufficient time and funding for long-term trials, or (2) have equipment and personnel so that shorter LRTs can be achieved.

Trials 3 and 4 had small VSLR and long LRT and were not run for a long time relative to the LRT. As a consequence, the total masses of reactant (NAVS) fed and product (carboxylic acid) removed were significantly less than the total initial or final mass in the fermentors. Because true steady state was not achieved, the difference in the initial and final amounts of reactant and product in the fermentors was significant. When comparing the performance of Trial 3 verse Trial 4, the overall performance of each trial must be calculated, which includes the initial and final content of each fermentor. Each parameter was calculated analogously as outlined in Section 4.4.5. Initial quantities were treated as "inlet streams" and final quantities were treated as "outlet streams." Table 4-4 summarizes the overall performance parameters for Trials 3 and 4.

Trials 3 and 4 both approximated steady state. The only operational difference between Trials 3 and 4 was the solid-liquid separation equipment. Trial 3 used an oilfield-style filter basket whereas Trial 4 used a Vincent KP-6 screw press. From Tables 4-3 and 4-4 it is clear that the filtration equipment influenced performance. Comparing Trial 4 to Trial 3, some parameters increased and others decreased. At first glance, it is not apparent which trial performed better. Both the "steady-state" and the overall performance show the same directional changes in performance, except for process yield.

Acid concentration

From Trial 3 to Trial 4, the total acid concentration of the product liquid (L_1;

liquid leaving F1) increased ~16% from 25.3 to 29.3 g acid/L_{liq}. F2 total acid concentration had no statistical change and F3 total acid concentration decrease ~15%. An identical trend was observed with aceq concentration. The acid concentration data for Trials 3 and 4 are plotted in Figures 4-8 and 4-9, respectively. Because the acid concentration in the filter liquids and solids moved on transfer day dictates influences yield, the transfer day acid concentrations are distinguished from daily measurements taken while the system was in batch.

To reestablish volume and solid-liquid composition set points during a material balance for a given fermentor, a net change of dry solids and solid-free liquid is required. The minimum exchange of transfer solids and liquids corresponds with a theoretical ideal filter that is 100% efficient (liquid-free filter solids and solid-free filter liquid). As the filter efficiency decreases, to achieve the same net transfer of dry solids and solid-free liquid, the amount of filter solids and liquids required increases; more of the opposing transfer stream is required to compensate for the non-ideal filtration. Conversely, as filtration efficiency increases, less filter solids and liquids are required for the same net exchange of dry solids and solid-free liquid.

By switching from the oilfield-style filter basket (Trial 3) to the Vincent KP-6 screwpress (Trial 4), the filter efficiency dramatically increased, which reduced back mixing. (For more details about filter efficiency see Section 2.1.) The observed increase in Fermentor 1 acid concentration results from less liquid being transferred from F2; thus less lower-acid-concentration liquid is transferred to F1 to dilute the acid concentration. Likewise, the explanation for the decreased acid concentration in F3 is identical; less transfer solids from F2, which contains higher-acid-concentration liquid, are transferred into F3 thereby artificially increasing the acid concentration.

Table 4-3. Summary of "steady-state" (ignores initial and final content in calculation) performance parameters for pilot-fermentation Trials 3 and 4. Error represents two standard deviations (95% confidence interval).

Parameter	Units	Trial 3	Trial 4	T4 vs. T3
Total acid conc., F1	g acid/L_{liq}	25.3 ± 1.4	29.3 ± 1.8	15.7%
Total acid conc., F2	g acid/L_{liq}	21.7 ± 3.3	21.7 ± 3.1	–0.1%
Total acid conc., F3	g acid/L_{liq}	17.4 ± 3.2	14.8 ± 4.2	–14.6%
Total aceq conc., F1	g aceq/L_{liq}	36.5 ± 1.7	42.9 ± 2.8	17.3%
Total aceq conc., F2	g aceq/L_{liq}	31.9 ± 5.2	32.2 ± 4.4	0.8%
Total aceq conc., F3	g aceq/L_{liq}	25.6 ± 4.7	22.6 ± 6.7	–11.6%
Feed yield, Y_F	g acid/g $NAVS_{feed}$	0.022 ± 0.00	0.020 ± 0.00	–5.8%
Exit yield, Y_E	g acid/g $NAVS_{feed}$	0.145 ± 0.00	0.137 ± 0.01	–5.3%
Culture yield, Y_C	g $acid_{produced}$/ g $NAVS_{feed}$	0.123 ± 0.00	0.117 ± 0.01	–5.2%
Process yield, Y_P	g acid/g $NAVS_{feed}$	0.104 ± 0.00	0.120 ± 0.01	14.9%
Total acid productivity	g $acid_{produced}$/(L_{liq}·d)	0.205 ± 0.01	0.176 ± 0.01	–14.1%
Conversion, C	g $NAVS_{consumed}$/ g $NAVS_{feed}$	0.527 ± 0.04	0.583 ± 0.05	9.6%
Selectivity, σ	g $acid_{produced}$/ g $NAVS_{consumed}$	0.233 ± 0.02	0.200 ± 0.02	–14.2%
closure (dry basis)	g out/g in	0.630 ± 0.20	0.564 ± 0.23	13.2%
closure (wet basis)	g out/g in	1.010 ± 0.20	0.744 ± 0.15	–26.3%

Table 4-4. Summary of overall performance parameters (includes initial and final content in calculation) for pilot-fermentation Trials 3 and 4. Error values could not be calculated.

Parameter	Units	Trial 3	Trial 4	T4 vs. T3
Feed yield, Y_F	g acid/g $NAVS_{feed}$	0.102	0.095	–6.6%
Exit yield, Y_E	g acid/g $NAVS_{feed}$	0.177	0.129	–27.0%
Culture yield, Y_C	g $acid_{produced}$/ g $NAVS_{feed}$	0.076	0.034	–54.4%
Process yield, Y_P	g acid/g $NAVS_{feed}$	0.153	0.123	–19.4%
Total acid productivity	g $acid_{produced}$/(L_{liq}·d)	0.219	0.127	–42.2%
Conversion, C	g $NAVS_{consumed}$/ g $NAVS_{feed}$	0.104	0.233	123.5%
Selectivity, σ	g $acid_{produced}$/ g $NAVS_{consumed}$	0.725	0.148	–79.6%
closure (dry basis)	g out/g in	1.031	0.804	–22.0%
closure (wet basis)	g out/g in	0.987	0.889	–10.0%

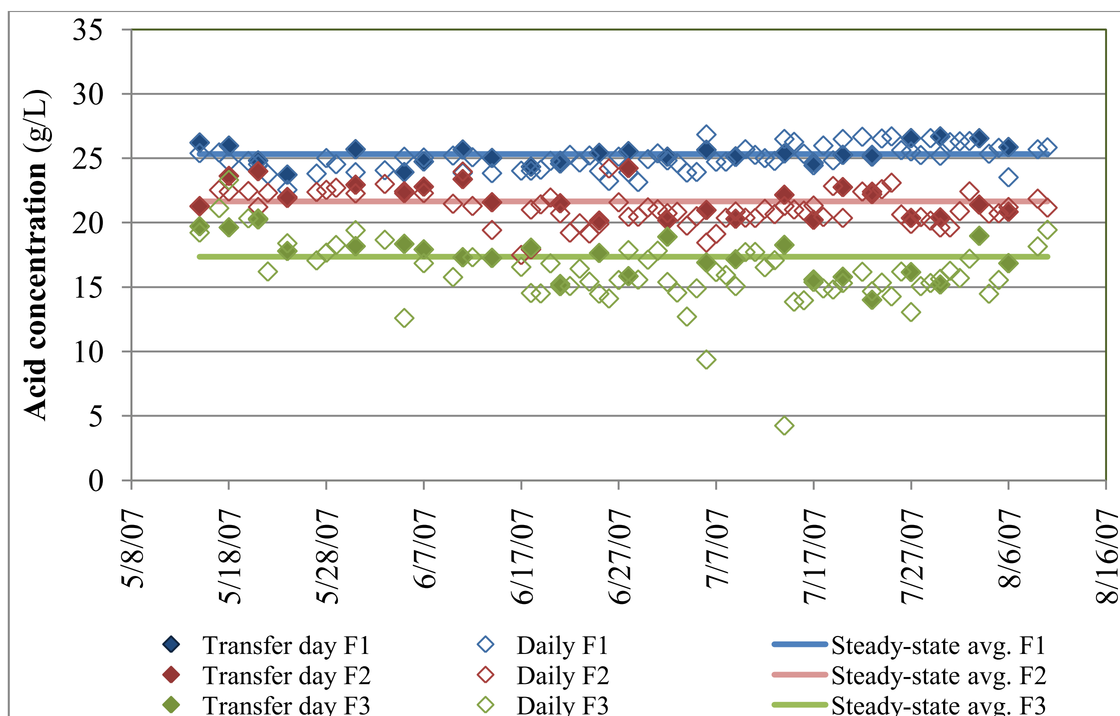


Figure 4-8. Pilot plant Trial 3 acid concentration for Fermentors 1–3 (F1–F3).

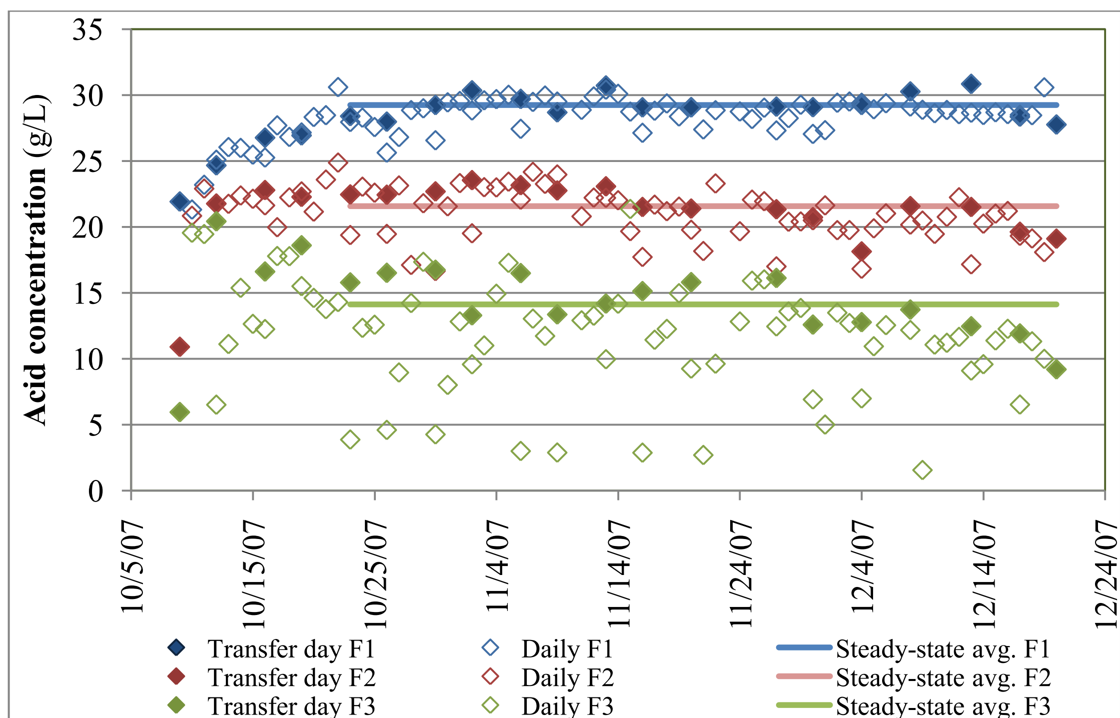


Figure 4-9. Pilot plant Trial 4 acid concentration for Fermentors 1–3 (F1–F3).

Feed yield

From Trial 3 to 4, the "steady-state" feed yield, which quantifies the ratio of acid to NAVS in the feed, decreased 5.8% from 0.022 to 0.020 g acid/g $NAVS_{feed}$. The change resulted from natural fluctuations in feedstock composition. The overall feed yield, which included the initial acid and NAVS present in all fermentors, decreased 6.6% from 0.102 to 0.095 g acid/g $NAVS_{feed}$. In addition to a reduced amount of acid in the feedstock, Trial 4 began with lower initial acid concentrations than Trial 3, thus the decrease in overall feed yield is larger than the "steady-state" value.

Exit yield, culture yield, and total acid productivity

The "steady-state" exit yield quantifies the mass ratio of acid leaving in the product liquid (L_1) and waste solid (S_3) per mass NAVS in feed. The overall exit yield includes the acid remaining in all fermentors in the numerator and the initial NAVS in all fermentors in the denominator. Both the "steady-state" and overall culture yields are the difference between the exit and feed yield for the respective calculation method, and thus quantify the net yield of acid *produced*.

The decrease in "steady-state" exit and culture yields occurred because the improved filter efficiency reduced back mixing, which decreased the acid concentration in F3 and the amount of solids removed from F3; thus, less acid left with waste solids. The dramatic decrease in overall exit and culture yields occurred because there was more acid in the fermentors at the end of Trial 3 than at the end of Trial 4, which is primarily attributed to the change in F3 acid concentration. The changes in "steady-state" and overall total acid productivity occurred because of the same effects previously described for exit and culture yield.

Process yield

The "steady-state" process yield quantifies the mass of acid in the product liquid (L_1) per NAVS in feed. The overall process yield includes acid remaining in all fermentors in the numerator and initial NAVS in all fermentors in the denominator. The process yield is important because it quantifies the yield relative to the product that is

sent to downstream processing.

For Trials 3 and 4, the "steady-state" process yield was 0.104 and 0.120 g acid/g NAVS feed, respectively, which is a 15% improvement. Both Trials had the same transfer frequency (2 transfers/week), product liquid removal rate (50 gal/transfer), and solids feed rate (~125 dry lb/transfer). Unlike the other performance parameters, the overall process yield has a directionally different change that the "steady-state" value. The overall process yield decreased 19.4% from 0.153 to 0.123 g acid/g NAVS, which occurred because there was more acid in the fermentors at the end of Trial 3 than at the end of Trial 4. Because (1) the product acid concentration had stabilized, (2) the same volume of product liquor was removed each transfer, and (3) the same amount of feedstock was added each transfer on a dry basis, the "steady-state" process yield closely approximates the steady-state process yield. Likewise, in Trials 3 and 4, the "steady-state" process yields were the best performance comparison.

Conversion

From Trial 3 to 4, "steady-state" conversion increased 9.6% from 0.527 to 0.583 g $NAVS_{consumed}$/g $NAVS_{feed}$. In contrast, the overall conversion increased 123.5% from 0.104 to 0.233 g $NAVS_{consumed}$/g $NAVS_{feed}$. Because the NAVS content of each fermentor and the waste solids stream (S_3) were increasing during both trials (i.e., not stable), the "steady-state" values are overstated. The "overall" values are an accurate representation of the *overall* conversion of each trial, but may be lower than would be observed if true steady state were achieved.

The decrease in conversion is incongruent with a decrease in culture yield. In is unexpected that less acid was produced during Trial 4 yet more NAVS (reactant) was consumed. It seems unlikely that increased filter efficiency would cause this to occur. The screwpress created filter soilds that were small crumbs and dry to the touch, whereas, the oilfield-style filter tanks produced large cake-like clumps that were wet to the touch. This physical change dramatically increased the surface area of the filter solid particles. It is possible that the increase in air-exposure increased aerobic activity thereby increasing conversion without increasing acid production. Although the

circumstances and data in Tables 4-3 and 4-4 strongly support this hypothesis, this is not a rigorous conclusion for the following reasons:

- Trial 3 began with four fermentors. Midway through the trial, it was converted to three fermentors. When the Trial-3 performance parameters were calculated, there were insufficient data to determine the initial contribution from the fourth fermentor.
- True steady state was not achieved.
- During each transfer, although the same volume of product liquid was removed and the same mass of feedstock was added, there are differences in the normalized operating parameters; thus, Trials 3 and 4 are not 100% comparable.

Selectivity

Selectivity is the ratio of acid *produced* per NAVS consumed. Mathematically, selectivity is equivalent to culture yield divided by conversion. From Trial 3 to 4, “steady-state” selectivity decreased 14.2% from 0.233 to 0.200 g $acid_{produced}$/g $NAVS_{consumed}$. Because the trials were not at steady state, the NAVS content of each fermentor increased as the ash content decreased (i.e., ash/sand purged from fermentors) therefore the “steady-state” conversions are overstated; thus, selectivity is understated.

The overall conversion selectivity decreased 79.6% from 0.725 to 0.148 g $acid_{produced}$/g $NAVS_{consumed}$. The apparent decrease in selectivity can be explained by the following:

- When the Trial 3 performance was calculated, there was insufficient data to quantify the initial contribution of the fourth fermentor. Thus, some of the acid that was associated with three fermentors was produced in the fourth fermentor; thus, the acid produced by three fermentors was overstated. Similarly, the amount of NAVS consumed by the fourth fermentor that produced that acid was unaccounted; thus, with respect to the acid produced, the NAVS consumed was underestimated. Because of these accounting issues, the selectivity of Trial 3 is overstated.

- Trial 4 had a lower culture yield because the improved filter efficiency reduced back mixing thereby reducing F3 acid concentration and amount of moisture, which contains acid that leaves with the waste solids. For reasons hypothesized above, Trial 4 had a higher conversion.
- Because of the high filtration efficiency and mechanics of the screwpress, the exiting filter solids were dry (0.50–0.7 g moisture/g total) crumbs that were typically less than 5–10 mm in diameter. In contrast, the filter tank filter solids were large wet clumps that could easily re-agglomerate. Because of the reduced moisture content and increased surface area, Trial 4 filter solids were more air-exposed. Air exposure could have exacerbated one or more of the following could, which could lead to low selectivity:
    1. acid degradation
    2. acid volatilization
    3. non-acid producing reactions (e.g., aerobic biomass decomposition)

Because of the accounting issues with Trial 3, rigorous comparison of all performance variables is not possible. Nonetheless, to understand trends and general phenomena, comparisons may be. Additionally, the above points explain bias; thus, overstated and understated values represent upper and lower limits, respectively, of the range of the true values.

Closure

The "steady-state" closure (dry basis) for Trials 3 and 4 were 0.63 and 0.56 g dry out/g dry in, respectively, which are lower than expected. In contrast the overall closure (dry basis) for Trials 3 and 4 were 1.03 and 0.804 g dry out/g dry in, respectively, which is expected. This discrepancy indicates that Trials 3 and 4 were not at steady state. The "steady-state" values appear low because the fermentors had a net gain of dry material over the trial period. When the initial and final amounts are accounted (overall closure) the closure is much closer to unity.

The decrease in overall closure could be a result of one or more of the following:

- *Increased gas production* – The pilot fermentors were not equipped to measure gas production, so this mass is unaccounted. Increased air exposure could have increase aerobic degradation thereby increasing carbon dioxide production.
- *Error* – Error cannot be determined for the overall calculation method; thus, it is possible that these closure values are statistically similar but there is insufficient data to make this conclusion.

4.5.2. CPDM predictions

Value agreement

For the three-stage pilot plant fermentation, the measured and CPDM-predicted acid concentrations and conversions are shown in Table 4-5. In general, the model overstated acid concentration and understated conversion. The conversion predictions were more accurate than the acid concentration predictions.

The "strict" CPDM F1 acid concentration predictions for Trials 3 and 4 were overstated by 86.9% (47.3 vs. 25.3 g acid/L) and 68.9% (49.5 vs. 29.3), respectively. To put these values in context, for the data shown in Figure 4-1, the greatest absolute error was 70%; thus, the "strict" acid concentration predictions are less accurate than has been observed with lab-scale validations. The "strict" CPDM conversion predictions for Trials 3 and 4 were understated by 11.0% (0.469 vs. 0.527 g $NAVS_{consumed}$/g $NAVS_{feed}$) and 16.3% (0.488 vs. 0.583 g $NAVS_{consumed}$/g $NAVS_{feed}$), respectively, which are similar to the average absolute conversion error (16.9 ± 17%).

The "relaxed" CPDM F1 acid concentration predictions for Trial 3 and 4 were overstated by 47.4% (37.3 vs. 25.3 g acid/L) and 34.8% (39.5 vs. 29.3), respectively. Although these predictions are more accurate than the "strict" predictions, they are *less accurate* that ~97% of all previous semi-continuous countercurrent laboratory CPDM predictions (Figure 4-1). The "relaxed" CPDM conversion predictions for Trials 3 and 4 were understated by 3.4% (0.509 vs. 0.527 g $NAVS_{consumed}$/g $NAVS_{feed}$) and 7.7% (0.538

vs. 0.583 g $NAVS_{consumed}$/g $NAVS_{feed}$), respectively, which are better than the average absolute error. The improved accuracy of the "relaxed" predictions clearly indicates that air-exposure *did* affect fermentation kinetic parameters. Additionally, comparison of the "strict" and "relaxed" predictions further reinforces that CPDM is highly dependent on the batch fermentations containing *ALL* chemical and biochemical components, both desired and undesired, that are present in the system being modeled.

Behavioral agreement

Ideally, the fermentation model would fully describe the chemical, biochemical, and physical phenomena so that predicted values and behaviors are accurate and reliable. Such a model is a very powerful design and optimization tool. However, if the model accurately mimics behaviors, even though the models values are inaccurate, the model can be used to understand trends, screen operating parameters, screen reactor configuration, and determine points of diminishing return, all of which are valuable optimization exercises.

Although there is significant error (>30% absolute) with the acid concentration predictions, the model does capture the following trends:

1. Trial 4 has higher acid concentration that Trial 3
2. The range of acid concentration is larger in Trial 4 than Trial 3
3. Trial 4 has higher conversion than Trial 3

The behavioral agreement with these trends is not a thorough validation of the model for use in trend and directional analysis, but it does provide evidence that the configuration model describes the physical aspects well.

Table 4-5. Comparison of pilot-fermentation acid concentration and "steady-state" conversion with CPDM predictions.

Performance Variable	Units	Measured value	Strict		Relaxed	
			CPDM	Error %	CPDM	Error %
TRIAL 3						
Acid Conc., F1	g acid/L_{liq}	25.3 ± 1.4	47.3	86.9%	37.3	47.4%
Acid Conc., F2	g acid/L_{liq}	21.7 ± 3.3	41.5	91.2%	29.7	36.9%
Acid Conc., F3	g acid/L_{liq}	17.4 ± 3.2	30.0	72.4%	19.7	13.2%
Conversion	g $NAVS_{consumed}$/ g $NAVS_{feed}$	0.527 ± 0.04	0.469	–11.0%	0.509	–3.4%
TRIAL 4						
Acid Conc., F1	g acid/L_{liq}	29.3 ± 1.8	49.5	68.9%	39.5	34.8%
Acid Conc., F2	g acid/L_{liq}	21.7 ± 3.1	41.3	90.3%	27.3	25.8%
Acid Conc., F3	g acid/L_{liq}	14.8 ± 4.2	26.1	76.4%	14.5	–2.0%
Conversion	g $NAVS_{consumed}$/ g $NAVS_{feed}$	0.583 ± 0.02	0.488	–16.3%	0.538	–7.7%

4.5.3. Reaction model - sources of error

Assumptions

Mixed-acid fermentations are complex reactions with numerous reactants, products, and catalysts (i.e., enzymes and microorganisms). A comprehensive mechanistic theory-based model is not practical. CPDM is an empirical model that generalizes fermentation rate as a function of conversion and acid concentration. Where there is insufficient data to empirically model known phenomena (e.g., selectivity is not constant) assumptions are made (e.g., selectivity is constant). The error contributed by each assumption is unknown. The following are key assumptions of the reaction model:

1. The acid production rate (Equation 4-1) can be accurately modeled by an empirical rate equation that is a function of conversion and acetic acid equivalents concentration (Equation 4-1).
2. The empirical rate constants can be accurately regressed from an array of batch fermentations with different initial NAVS concentrations and/or acid concentrations.
3. Acid selectivity is constant. The constant value is typically obtained from (1) a countercurrent fermentation using the same substrate system or (2) the CPDM batch fermentations.
4. Product spectrum (i.e., distribution of different molecular weight acids) is constant (i.e., φ and aceq ratio are constant). The constant value is typically obtained from (1) a countercurrent fermentation using the same substrate system or (2) the CPDM batch fermentations.
5. There are no nutrient limitations.
6. Constant temperature.

Assumptions 3 and 5 likely contribute the greatest error. Because the current batch fermentation procedure does not measure conversion with respect to time; the selectivity may be measured only at the end of the batch fermentation. No research has

investigated the parameters that regulate selectivity. Aiello-Mazzarri (2002) correlated selectivity as a function of VSLR; however, this is a symptomatic approach and does not elucidate the fundamental driving forces. Data presented in Sections 6 and 8 suggest that selectivity is a function of nitrogen content (total or soluble nitrogen) and acid concentration.

Nutrients clearly influence fermentation kinetics. Nitrogen and phosphorus are essential for bacterial growth and enzyme production (e.g., hydrolysis enzymes such as cellulase). Without nutrients containing these elements, acid production will be limited regardless of the conversion and acid concentration. A unique phenomenon of countercurrent fermentations is nutrient distribution among stages is dictated by the VSLR, LRT, and stream moisture contents. Depending on the operating parameters and nutrient feed point(s), a given stage may have excess or limiting nutrient concentrations. (For more details, see Sections 6 and 7.)

Because the specific rate equation is not a function of nutrient content and nutrient distribution is not even in a countercurrent fermentation, Assumption 2 may overestimate fermentation kinetics. In batch fermentation, nutrients cannot be flushed out, which can occur in a countercurrent system. The nutrient concentrations and/or proportions in a batch system are most likely not the same as will occur in the countercurrent fermentation. Thus, the kinetics observed in batch fermentations may or may not be applicable to countercurrent fermentations. The discrepancy is likely directly related to the absolute and/or relative throughput rates of feedstock and water, which is an observation made by Aiello-Mazzarri (2002).

Because the pilot plant was an open-air facility, the fermentors were subject to weather variations. Although the fermentors were insulated and were equipped with heaters, the fermentor temperature fluctuated 5–10 °C from the set point (40 °C). As a rule of thumb, reaction rates double every 10 °C; thus, temperature fluctuation could have contributed error or averaged out over time.

In addition to error related to underlying kinetic assumptions, the following are potential *significant* sources of error: (note: This is not an exhaustive list. It is beyond

the scope of this section to identify *all* error sources.)

- *Air-exposure* – Comparison of the "strict" and "relaxed" CPDM predictions clearly shows that incorporating air-exposure in the batch fermentations improved accuracy. It is possible that the CPDM batch fermentations were not air-exposed to an equivalent extent as the pilot plant fermentations. Perhaps further air exposure would have improved prediction accuracy.
- *Gas and water production* – The current modeling frame work only considers acetic acid equivalents as fermentation product. There are two errors with this: (1) Acetic acid equivalents (aceq) do not represent the true mass of acid; thus, when accounting for the mass of NAVS consumed in the program, it is inappropriate to subtract the mass of aceq produced. The aceq mass must be converted to acid mass via the acetic acid equivalence ratio. (2) Considering only aceq (or acid) as a product fails to account for gas (carbon dioxide and hydrogen) and water as fermentation products.
- *Unbounded aceq concentration* – The specific rate equation (Equation 4-1) does not have an embedded boundary condition for aceq concentration as it does for conversion. At a conversion of 1 g $NAVS_{consumed}$/g $NAVS_{feed}$ the specific rate is zero. However, assuming conversion is less than 1 g $NAVS_{consumed}$/g $NAVS_{feed}$, the aceq concentration must be infinity for the rate to go to zero. The expression of aceq concentration should be changed so that a boundary condition is embedded within the specific rate equation. For example, consider Equation 4-23, which is a modified expression of Equation 4-1 (modified term is **bold**).

$$\hat{r} = \frac{e\,(1-x)^{f}}{1 + g\left(\dfrac{\boldsymbol{A_e}}{\boldsymbol{A_{e,\,max} - A_e}}\right)^{h}} \qquad (4\text{–}23)$$

where $A_{e,max}$ is the maximum measured aceq concentration for a substrate system. Note: φ was eliminated as discussed in Section 4.3.1.

With Equation 4-23, assuming conversion is less than 1 g $NAVS_{consumed}$/g $NAVS_{feed}$, as the aceq concentration approaches $A_{e,max}$ the denominator goes to infinity thereby forcing the rate to zero. If $A_{e,max}$ was determined from the CPDM batch fermentations, use of Equation 4-23 would prevent the model from extrapolating the data set, which was the case for the predictions shown above. The maximum observed acid concentrations for the "strict" and "relaxed" CPDM batch fermentations were 28.4 and 30.0 g acid/L, respectively. The max acid concentration for the "relaxed" CPDM batches *was* higher than the "strict" CPDM batch. The highest instantaneous pilot plant acid concentration was <32 g acid/L.

4.5.4. Configuration model – sources of error

Compared to the reaction model, the configuration model more closely represents actuality and contains less significant assumptions. The following are key assumptions of the configuration model:

1. System is continuous and not semi-continuous (i.e., cycle of batch operation followed by material transfer).
2. Holdup of transfer solids (i.e., cake) is constant for all solid streams.
3. Transfer liquid does not contain solids.
4. Ideal mixing; no spatial variations.

Although these assumptions do not completely reflect reality, they are a good approximation and are unlikely to contribute significant error (>10% error). The configuration model could be more accurate by increased accounting of the solids and liquids in each stream.

In addition to error related to these configuration assumptions, the following are potential *significant* source of error:

- *Non-ideal operation* – During Trial 3, the following summarize events that deviated from the intended mode of operation, all of which are not captured by

the computer model.

- Leakage from sludge pumps
- Transition from a four-fermentor train to a three-fermentor train
- Water addition to a fermentor other than last fermentor to lower the percent solids so that the slurry can be pumped.

- *True steady-state not achieved* – Because the LRTs were so long, Trial 3 and 4 were both run for less than half a LRT. Typically, continuous systems must be run for 3–10 LRTs before steady state is achieved. Although the acid concentration *appeared* to have stabilized, it is possible that acid concentration would have continued to increase if the fermentation was continued for multiple LRTs.

4.6. Conclusions

- Steady state *can be* achieved in pilot-scale submerged fermentations; the steady-state strategy is robust.
- Although acid product concentration had stabilized, neither Trial 3 nor Trial 4 achieved true steady state. Steady state should be determined by evaluating all process variables – not just acid concentration.
- Trial 4 was a better approximation of steady state than Trial 3.
- Trial 4 performed better (i.e., greater F1 acid concentration, and larger "steady state" process yield) Trial 3.
- Future pilot plant fermentation trials should (1) allocate time and funding to have long-term trials or (2) have equipment and personnel so that shorter LRTs can be achieved.
- Dewatering efficiency directly effects performance because back mixing is reduced and less acid is lost with waste transfer solids.
- CPDM batch fermentations must contain both intended (e.g., paper, chicken manure, buffer, N supplement) and unintended (e.g., air, oxygen) chemical and

biochemical reactants that *are* present in the modeled system.

- The relaxed anaerobic CPDM predictions were more accurate than the strict anaerobic CPDM predictions.
- Air-exposure subdued fermentation kinetics (i.e., acid production rate was reduced).
- The most significant errors are likely related to the reaction model.
- The CPDM predictions overstated acid concentration, which was an extrapolation of the batch data used to regress the empirical constants of the specific reaction rate.
- Mixing is important, but continuous vigorous mixing is not necessary. For the pilot fermentation, one hour of mixing (manual mixing combined with operating fermentor recycle loop) was sufficient to perturb less-active areas and disperse methane inhibitor.
- Accurate material balances are critical to achieve steady state; thus, improving dry solid concentration (dry lb/bulk gallon) measurement is critical to the success of submerged fermentations.
- Mathematically, φ is unnecessary; thus, to simplify Equation 4-1, φ should be eliminated.

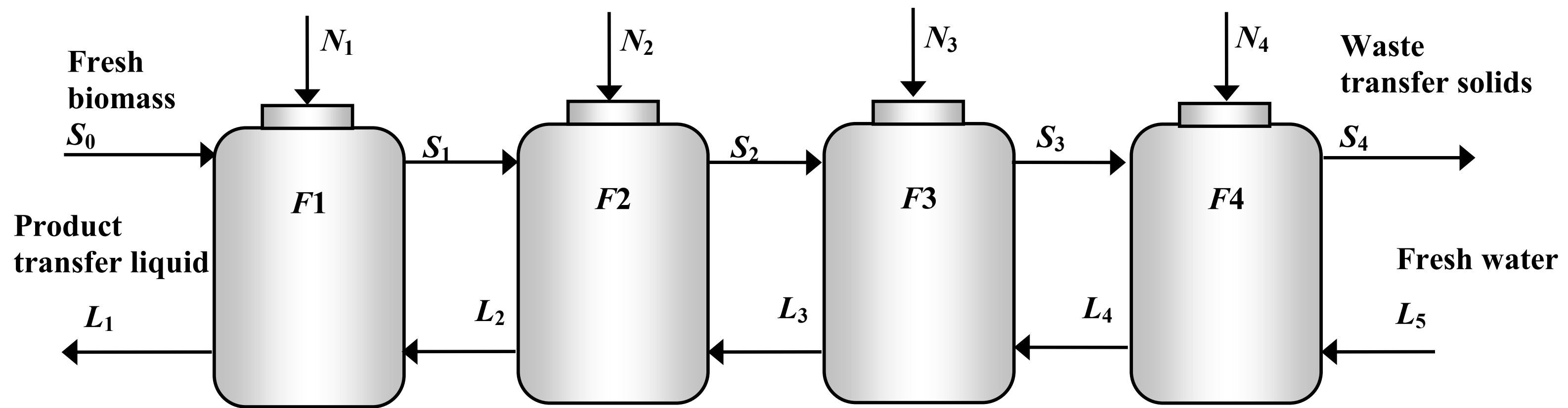
5. THE SLOPE METHOD: A TOOL FOR ANALYZING SEMI-CONTINUOUS DATA

The MixAlco^{TM} process is a biorefinery that converts lignocellulose into useful chemicals and hydrocarbon fuels via mixed-acid fermentation. For a semi-continuous staged fermentation train, during each transfer, discrete amounts of material are moved between fermentors and data are tabulated. Because of natural day-to-day variations, the data are inherently noisy. To calculate performance parameters (e.g., yield, conversion, selectivity, productivity), the average flowrate of each stream component must be determined. To minimize error associated with noise, three data analysis methods were compared: Average, Accumulation, and Slope. The Average method determines the flowrate by averaging the amounts moved each transfer. The Accumulation method stores the solids and liquids that exit the fermentation train in separate vessels. After an extended time period the mass in each storage vessel is measured so the average flowrate can be calculated. The Slope method calculates the flowrate of material in each stream from the slope of the moving cumulative sum with respect to time. For all three methods, the measured rates were virtually identical; thus *accuracy* was not affected by the method. However, the Average method had >40% error and the Slope method <2% error; thus, *precision* was significantly affected by the method. The Accumulation method calculated the flowrate with a single data point so it is not possible to determine the error.

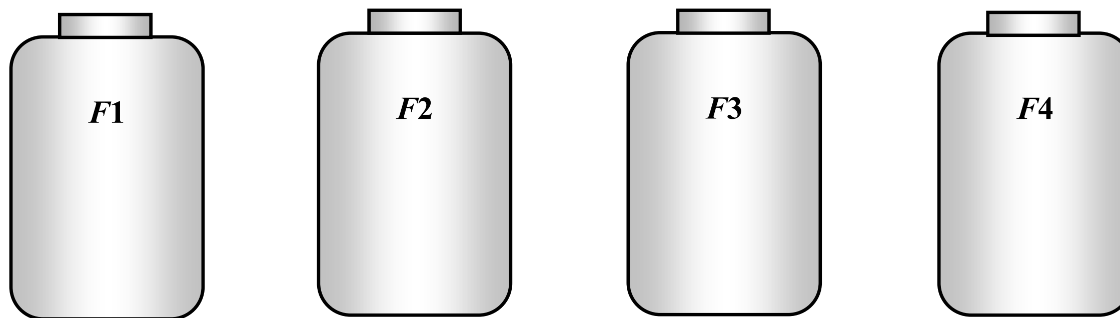
5.1. Introduction

The MixAlco^{TM} process is a biorefinery that converts lignocellulose into useful chemicals and hydrocarbon fuels via mixed-acid fermentation (Holtzapple and Granda, 2009; Holtzapple et al., 1999; Smith et al., 2010). In the laboratory and pilot plant, countercurrent staged mixed-acid fermentations are operated in a semi-continuous fashion (Figure 5-1); every two or three days, a discrete amount of fermentation material is removed from each fermentor and filtered. Mass balances determine the amounts of filtered solids and liquids that are transferred in opposite directions to the respective adjacent fermentor (Figure 5-1A). Between countercurrent transfers, the individual fermentors operate in batch mode (Figure 5-1B).

To monitor and measure the performance of the semi-continuous fermentation, the stream masses from each countercurrent transfer are tabulated. These discrete quantities may be raw data (e.g., total stream mass or volume) or calculated component quantities (e.g., liquid, dry solid, non-acid volatile solids (NAVS), ash, or carboxylic acid (product) masses). Fermentation performance values (e.g., yield, conversion, productivity) are calculated from steady-state average rates of these stream components. Because the semi-continuous data can be very noisy, minimizing error when calculating the average steady-state component rates is a challenge.



(A)



(B)

Figure 5-1. (A) Countercurrent movement of material during a transfer. *Si*, *Li*, and *Ni* represent the transfer solid, transfer liquid, and nutrient streams, respectively. *Fi* represents the fermentations mass in Fermentor i. (B) Batch operation between transfers.

Historically, to determine steady-state component flowrates, the Average and Accumulation methods have been used. The *Average method* determines the flowrate by averaging the amounts moved each transfer, which represent instantaneous rates (i.e., differentiated data). As a consequence, very large errors make statistically meaningful conclusions difficult. The *Accumulation method* physically stores the filtered solids and liquid exiting the fermentor train in a storage vessel. After an extended time period (i.e., one or two months), the accumulated mass in the storage vessel is measured resulting in a single data point. It does not provide sufficient data to calculate error; thus, statistically meaningful comparisons are impossible. The *Slope method* is a recent innovation that measures the stream rate and error from the data *trend*. In all three methods, the average stream flowrate is theoretically identical; however, the objective is to dramatically reduce the error so that statistically meaningful comparisons can be made between two different fermentation trains.

5.1.1. Slope-based methods

When analyzing data, slopes are commonly used to determine rates; however, the correct method is not always apparent and has been the subject of research. For example, Michaelis-Menten parameters are determined from the slope and intercept of a Lineweaver-Burk plot, which was a refinement of previous technique (Donefer et al., 1960; Hurwitz, 1964; Kunz and Leeuw, 1993; Sue et al., 1988; Thomas, 1937; Trevelyan, 1966). A few literature articles explicitly develop a “slope method” to determine a *rate*. To simplify what was previously a laborious calculation, Thomas (1937) developed a slope method to determine the reaction rate constant of polluted water; however, his slope method was understood to be less accurate than the traditional method. Hurwitz (1964) showed that phosphorus uptake in baby chickens, which is a semi-continuous process, is linear and the slope determines the rate. To determine the rate yeast uptake sugar, Trevelyn (1966) applied a material balance around a continuously fed fermentor and measured the rate of change of the extracellular sugar concentration from the slope of an appropriate plot.

In the above-referenced articles, the focus of the "slope methods" was the *value* of interest. In contrast, the motivation for the Slope method presented this paper is *error reduction*. The Slope method is further differentiated because of its application to semi-continuous fermentations and its use of a moving cumulative sum.

5.2. Methods

To demonstrate and compare the Average, Accumulation, and Slope methods, the example data (Smith et al., 2011) shown in Table 5-1 lists the mass removed from Fermentor 4 (S_4) during each transfer. All stream rate calculations were made over the steady-state region (Days 76–162). In practice, aspects of these methods have been combined (e.g., Average method used to determine total stream flowrate; Accumulation method used to determine a component rate of the same stream); however, in this paper each will be discussed and evaluated as a separate method so that the advantages and disadvantages are apparent. Unless otherwise stated, "error" refers to two standard deviations.

5.2.1. Average method

In the Average method (Agbogbo and Holtzapple, 2006; Aiello-Mazzarri et al., 2006; Chan and Holtzapple, 2003; Domke et al., 2004; Thanakoses et al., 2003), samples are taken from the inlet and outlet streams to measure properties (e.g., moisture content, acid concentration, ash content) so that the mass of each stream component may be determined. Implementing the Average method requires that data be collected and samples analyzed upon each transfer, which can be a logistical challenge.

Equations 5-1 and 5-2 mathematically describe the Average method. For a given transfer period i, dM_i/dt is the stream rate of Material M.

Table 5-1. Example countercurrent transferred stream data (Section 6 – Train 2, Stream *S*4).

A	**B**	**C**	**D**	**E**	**F**	**G**
Row #	Date	Time of day	Nominal time	Exact time	Material transferred	Σ Material transferred
			(day)	(day)	(g)	(g)
1	**10/23**	16:17	0	0.00	26.3	26.3
2	**10/25**	10:56	2	1.78	79.1	105.4
3	**10/27**	13:45	4	3.89	129.1	234.5
4	**10/29**	16:18	6	6.00	196.7	431.2
5	**10/31**	16:45	8	8.02	166.0	597.2
⋮	⋮	⋮	⋮	⋮	⋮	⋮
65	**3/23**	10:17	151	150.75	168.5	12013.6
66	**3/25**	16:52	153	153.70	196.4	12210.0
67	**3/27**	17:09	155	155.71	96.0	12306.0
68	**3/30**	9:34	158	158.40	138.8	12444.8
69	**4/1**	18:02	160	160.75	152.8	12597.6
70	**4/3**	17:44	162	162.74	204.5	12802.1
				average	159.5	g/transfer
			2 standard deviations		79.9	g/transfer

$$\frac{dM_i}{dt} = \frac{M_i}{t_i - t_{i-1}} = \frac{M_i}{T_i} \tag{5-1}$$

where,

M_i = amount of Material M transferred

t_i = time when Transfer i was executed.

$T_i = t_i - t_{i-1}$ = time since the previous transfer

The Average method assumes the transfer period T is constant. Thus, the average steady-state stream rate ($d\overline{M}/dt$) is determined according to Equation 5-2.

$$\frac{d\overline{M}}{dt} = \frac{\sum_{i=1}^{N} M_i}{nT_i} \tag{5-2}$$

where,

n = number of transfers (i.e., data points) during steady state

Figure 5-2A illustrates the Average method, where the horizontal solid line represents the average mass removed per transfer. The steady-state stream or stream component flowrate is a simple arithmetic average of the material per transfer (i.e., steady-state average of Column F in Table 5-1, multiplied by the appropriate conversion factor (e.g., average transfer frequency; transfers/day) so that the flowrate is computed in the desired time units (typically per day). To determine the error of the average, the standard deviation of the data set (Table 5-1, Column F) is calculated.

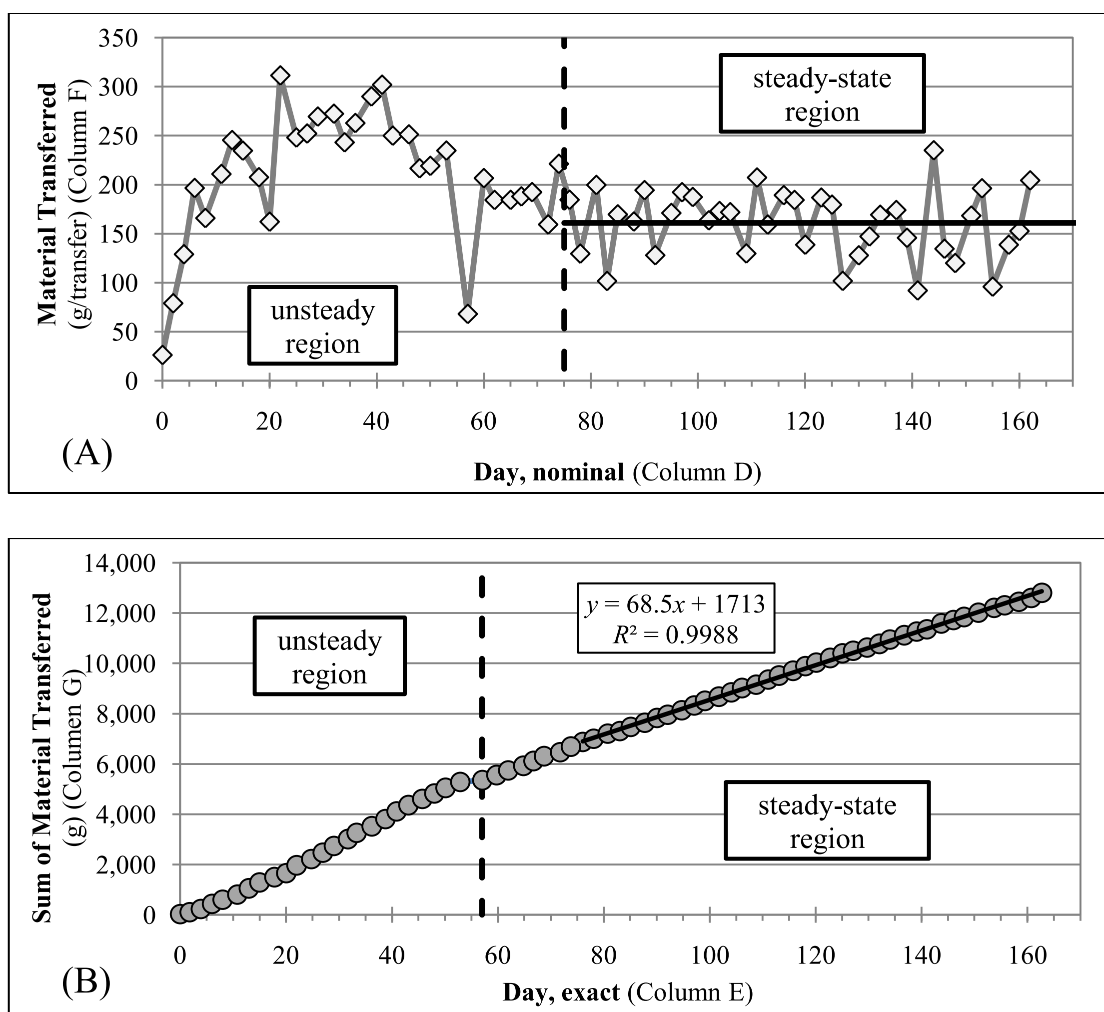


Figure 5-2. (A) Stream material moved each transfer with time (Average method); (B) Moving cumulative sum of stream material transferred with time (Slope method). Solid line represents steady-state trends from Days 76–162. Dashed line represents the beginning of an observable steady-state region using the respective method.

5.2.2. Accumulation method

In the Accumulation method (Agbogbo and Holtzapple, 2006; Aiello-Mazzarri et al., 2006; Chan and Holtzapple, 2003; Domke et al., 2004; Thanakoses et al., 2003), solids and fluids exiting the fermentation train are *accumulated* in a container once

steady state begins. The accumulated material is frozen and analyzed at the end of the fermentation. Because only *one* measurement is used to determine the average flowrate and stream component masses, it is impossible to determine the error. An additional limitation is that the Accumulation method can only be applied to outlet streams (i.e., product transfer liquid, and waste transfer solids). Although it can be used to determine the *overall* performance of the fermentation train, it cannot be used to evaluate the performance of a *single* fermentor.

Mathematically, the accumulation method (Equation 5-3) is similar to Equation 5-1. The numerator is the sum of Material *M* transfer, but the individual masses are unknown. The denominator is the length of the steady state period.

$$\frac{d\overline{M}}{dt} = \frac{\sum M_i}{t_{SS\,end} - t_{SS\,start} + 1} \tag{5-3}$$

5.2.3. Slope method

The Slope method, illustrated in Figure 5-2B, determines the average flowrate based on the data *trend* rather than averaging discrete transfer quantities. The amount transferred M_i and time are treated as independent variables. To determine the average flowrate, the average mass of *M* transferred and average transfer time period are calculated separately then divided, as shown in Equation 5-4.

$$\frac{d\overline{M}}{dt} = \frac{\dfrac{\sum_{i=1}^{n} M_i}{n}}{\dfrac{\sum_{i=1}^{n} (t_i - t_{i-1})}{n}} \tag{5-4}$$

Adjusting time such that steady state starts at $t_0 = 0$, Equation 5-4 may be simplified and rearranged into a "$y = m \cdot x$" form.

$$\sum_{i=1}^{n} M_i = \frac{d\overline{M}}{dt} \cdot t \tag{5-5}$$

Equation 5-5 shows that the average flowrate of Material M may be determined from the slope (not the y-axis value; Figure 5-2) of the moving cumulative sum of M_i with respect to time (Table 5-1; Column G).

Equations 5-6 – 5-11 (Skoog et al., 1996) outline the regression formulas used to calculate the slope m and the standard deviation D_m of the slope.

$$m = \frac{S_{xy}}{S_{xx}} \tag{5-6}$$

$$D_m = \sqrt{\frac{D_r^2}{S_{xx}}} \tag{5-7}$$

where,

x_i = time data

y_i = moving-cumulative-sum data

N = number of (x_i, y_i) data points

$$S_{xy} = \sum x_i\, y_i - \frac{\sum x_i \sum y_i}{N} \tag{5-8}$$

$$S_{xx} = \sum x_i^2 - \frac{(\sum x_i)^2}{N} \tag{5-9}$$

$$S_{yy} = \sum y_i^2 - \frac{\left(\sum y_i\right)^2}{N} \tag{5-10}$$

$$D_r = \sqrt{\frac{S_{yy} - m^2 S_{xx}}{N - 2}} \tag{5-11}$$

Zellmer (1997) states that S looks like a variance; however, it is just the sums of squares of the deviations of x (or y) from the mean of x (or y).

In Microsoft Excel, the slope m and standard deviation of the slope D_m may be calculated using Equations 5-12 and 5-13.

$$m = \mathbf{SLOPE}(Y_i, X_i) \tag{5-12}$$

$$D_m = \mathbf{SQRT}(\mathbf{STEYX}(Y_i, X_i)\text{^}2/((\mathbf{COUNT}(Y_i)-1)*\mathbf{STDEV}(X_i)\text{^}2)) \tag{5-13}$$

where, X_i and Y_i are the x_i and y_i data arrays, respectively, and the Microsoft Excel functions are in bold.

The Slope and Accumulation methods are similar except for the time period over which the accumulation is done. In the Slope method, the accumulation period is the shortest possible (i.e., each transfer). In the Accumulation method, the accumulation period is the longest possible (i.e., the entire steady-state period). If logistics (e.g., limited labor relative to the large number of fermentation trains) prohibit the analysis of exit streams after *each* transfer, then one could compromise and accumulate over a set time period such as one week (e.g., three transfers).

5.2.4. Order of calculations

In the Slope method, when using a spreadsheet to calculate performance parameters, the following order of operations should be followed:

1) From the raw data (e.g., time, total stream mass, moisture content, acid concentration), calculate the mass of each stream component (e.g., acid, NAVS, dry solid) for each transfer.
2) For each stream component, add a column in which to calculate the moving cumulative sum.
3) Using the steady-state moving cumulative sum, time, and Equations 5-6 – 5-13, calculate the component flowrate and error.
4) Calculate the desired performance parameters (e.g., yield, conversion, selectivity, productivity) from these steady-state rates.
5) Use sum-of-squared errors techniques (Section 5.2.5) to determine the propagation of errors for the desired performance parameters.

5.2.5. Sum of squared errors

Addition and subtraction

When two or more values that have errors are added and/or subtracted in a single term, Equation 5-14 may be used to determine the error.

$$\delta_Z = \sqrt{\delta_A{}^2 + \delta_B{}^2 + \ldots} \qquad (5\text{-}14)$$

where,

δ_Z = final error of the sum and/or difference

δ_i = error of Value i

For example, $(3.2 \pm 0.3) + (12.1 \pm 1.1) - (6.1 \pm 0.8) = 9.2 \pm 1.4$.

Multiplication and division

When two or more values that have errors are multiplied and/or divided in a single term, Equation 5-15 may be used to calculate the error.

$$\delta_Z = Z \cdot \sqrt{\left(\frac{\delta_A}{A}\right)^2 + \left(\frac{\delta_B}{B}\right)^2 + \ldots} \qquad (5\text{-}15)$$

where,

δ_Z = final error of the product and/or quotient

Z = the final value of the product and/or quotient

δ_i = error of Value i

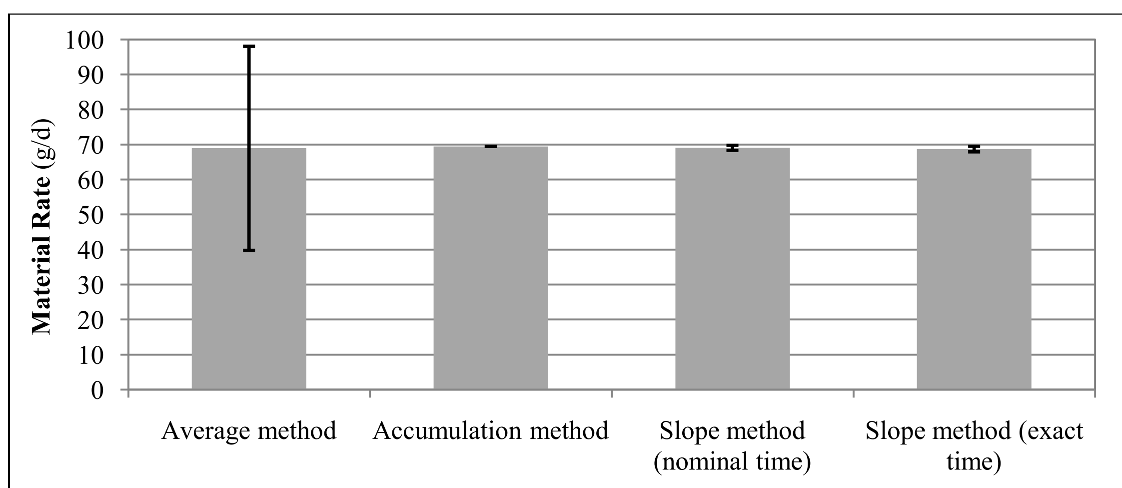
A, B, etc. = the value of Value i

For example,

$$\frac{(3.2 \pm 0.3) \cdot (12.1 \pm 1.1)}{6.1 \pm 0.8} = 6.3 \pm 0.2$$

5.3. Results and discussion

Figure 5-3 shows that the Average, Accumulation, and Slope methods result in virtually identical flowrates; thus, *accuracy* is not significantly affected by the method. Therefore performance parameters based on rates determined using any of these methods will be similar. However, the error calculated by each method is dramatically different. For the example data, the Average and Slope methods had an error of ~42% and ~1%, respectively. The Accumulation method cannot determine error. Sections 5.3.1–5.3.3 evaluate the mechanics, advantages, and disadvantages of each method.



	Average Method	**Accumulation Method**	**Slope Method** (nominal time)	**Slope Method** (exact time)
Material flowrate (g/d)	68.9	69.5	69.1	68.5
2 standard dev.	29.1	n/a	0.72	0.80
Error (%)	42.3	n/a	1.0	1.2

Figure 5-3. Comparison of methods for determining stream material flowrates. Error bars represent two standard deviations (95% confidence interval). For comparison, all flowrates are evaluated over the same time period, Days 76–162.

5.3.1. Average method

The Average method has large error (Figure 5-3) because it averages instantaneous data (i.e., discrete transfer amounts) that are noisy. Ideally, transfers would be conducted precisely on a constant time interval; however, in practice, this is not always logistically possible. The Average method falsely assumes that the time associated with each transfer is constant (Table 5-1; Column E); thus, each transfer amount is weighted equally with respect to time. Because the fermentation is active while in batch mode, an increase in the time interval between transfers causes the amount of each stream component to increase (e.g., carboxylic acid; product) or decrease (e.g., NAVS; reactant), thus resulting in noisy instantaneous data. Additionally, operator error, equipment, and feedstock fluctuations contribute variability.

5.3.2. Accumulation method

The Accumulation method is logistically the simplest, and greatly reduces the volume of data and analysis required. However, it has several key disadvantages that make it the least rigorous method. Because the accumulated material is quantified and characterized at the end of a steady-state period, only one data point is taken; thus, error cannot be calculated nor can statistical comparisons be made.

Because component stream masses are not quantified upon each transfer, acid concentration is the only data from which to judge the onset of steady-state operation (i.e., when sample accumulation begins). Although acid concentration is a good indicator of steady state, using it as the *sole* parameter fails to evaluate the system as a whole. The transition from unsteady to steady operation is not readily apparent until well after steady-state has been established (see Figure 5-2). If accumulation of material is initiated prematurely, the presumed steady-state measurement will be corrupted. Unlike the Average and Slope methods, the Accumulation method does not provide sufficient data to adjust the steady-state time region during post-experiment analysis so that the most stable data set is evaluated.

5.3.3. Slope method

Figures 5-2 A and 5-2B clearly demonstrate that the Slope method effectively smoothes semi-continuous data and reduces error because it calculated flowrates based on the data *trend* rather than averaging instantaneous rates. Because the data are smoother, the transition from unsteady to steady-state operation is more apparent. For the example data, the Slope method allows a longer steady-state period to be observed (Figure 5-2B), which help demonstrate control on a large-scale fermentation train, or satisfy steady-state operation time requirement (e.g., two retention times).

To determine if error in the Slope method can be reduced, the component flow rates were calculated using the exact transfer time and nominal transfer time. For the example data (Table 5-1), the error slightly increased when the exact time was used; however, both errors are very similar. It is the authors' opinion that the flowrate determined using the exact time is more accurate and should be used if the data are available.

5.3.4. Application to large-scale operations

When operating a semi-continuous large-scale fermentation (e.g., pilot, demonstration, and commercial plants), the Slope method can be used to provide feedback and manage large fluctuations in transferred stream masses. To control the volume and dry solids content, large-scale staged fermentations require material balances be performed prior to each transfer, from which the discrete transfer stream masses are calculated. To provide the necessary inputs for material balances, regular (e.g., daily) volume and dry solid content measurements must be taken. For large-scale fermentations, accurate and precise measurement of the dry solid content is challenging because of biomass stratification, gas lift, limited sampling ports, mixing limitations that prevent homogeneous sampling, and logistics. These issues amplify noise in the semi-continuous data, which severely affects the material balance calculations causing large swings in transfer masses, which can upset steady state and place undue burdens on equipment and labor.

After starting a large-scale operation, the Slope method can be used to smooth noisy data so that an average total stream mass rate can be established, which can then be used to cross check the discrete stream masses dictated by the material balance. For example, assume the average stream flowrate is 100 kg/d and the transfer frequency is once every two days; thus, approximately 200 kg/transfer is expected. Based on this average flowrate, to counteract data volatility, operators may establish practical limits (e.g., minimum = 100 kg/transfer; maximum = 300 kg/transfer) that maintain operation within the limits of available equipment and labor. Thus, if a material balance calls for 500 kg to be transferred, the Slope method provides insight that this amount *might be* erroneous so the maximum (300 kg/transfer) should be substituted. If steady-state operation truly requires larger transfer amounts (i.e., >200 kg/transfer), a consistent trend will then be observed so that appropriate adjustments can be made. Thus, when using the Slope method to guide operations, the data array should be updated frequently so the most recent and steadiest data are used.

Although the Slope method can be used to establish trends that help stabilize transfer stream flowrates, the actual stream flowrates should always be determined from material balances. Additionally, the Slope method does not determine when steady state is achieved; it only makes the data smoother so the transition from unsteady to steady state is more apparent. For most semi-continuous fermentations, it is not necessary to know when steady state begins in real-time. However, when analyzing data, it is critical to accurately identify the beginning of steady state so the most representative data are used to calculate performance parameters.

5.3.5. Fermentation stability

Although mixed-acid fermentation has been shown to be robust with respect to product contamination and adaption to feedstock fluctuations (Domke et al., 2004; Holtzapple and Granda, 2009; Holtzapple et al., 1999; Sierra et al., 2008), some may view the complexity (i.e., undefined reactants, undefined reaction pathways, dirty, mixed cultures) of the mixed-acid fermentation reactions as a liability citing that it is

unpredictable and difficult to control. The Slope method shows that the semi-continuous mixed-acid fermentations are much more stable that previously observed. The level of noise observed with example data is typical of most stream components in mixed-acid semi-continuous staged fermentations. Some of the noise can be linked to operational non-idealities (e.g., imprecise transfer frequency, feedstock and/or nutrient fluctuations, measurement error, and fluctuations in solid-liquid separation); thus, not all of the error is indigenous to the fermentation reactions.

5.4. Conclusions

For semi-continuous fermentations, the Average, Accumulation, and Slope methods produce the same average flowrates. The Accumulation method produces only a single measurement, so it is not possible to determine the statistical error. The Slope method has minimal error (< 2%) compared to the Average method, which has much larger error (> 40%). The Slope method is useful for analyzing data to determine fermentation performance parameters (e.g., yield, conversion, selectivity, productivity). It can also be used to stabilize stream flowrates of large-scale fermentations. The Slope method shows that semi-continuous mixed-acid fermentations are very stable and consistent.

6. INVESTIGATION OF NUTRIENT FEEDING STRATEGIES IN A COUNTERCURRENT MIXED-ACID MULTI-STAGED FERMENTATION: EXPERIMENTAL DATA

Nutrients are essential for microbial growth and metabolism in mixed-culture acid fermentations. Understanding the influence of nutrient feeding strategies on fermentation performance is necessary for optimization. For a four-bottle fermentation train, five nutrient contacting patterns (single-point nutrient addition to Fermentors F1, F2, F3, F4, and multi-point parallel addition) were investigated. Compared to the traditional nutrient contacting method (all nutrients fed to F1), the near-optimal feeding strategies improved exit yield, culture yield, process yield, exit acetate-equivalent yield, conversion, and total acid productivity by approximately 31%, 39%, 46%, 31%, 100%, and 19%, respectively. There was no statistical improvement in total acid concentration. The traditional nutrient feeding strategy had the highest selectivity and acetate-equivalent selectivity. Total acid productivity depends on carbon-nitrogen ratio.

6.1. Introduction

6.1.1. The MixAlco^{TM} process

The MixAlco^{TM} process (Figure 1-3) is a "biorefinery" that converts any biodegradable biomass into useful chemicals and fuel (Holtzapple and Granda, 2009; Holtzapple et al., 1999). Although some substrates (e.g., food scraps and office paper) are easily digested, most lignocellulosic biomass must be pretreated with lime and oxygen/air to increase digestibility. The biomass is then fermented by a mixed culture of acidogens to produce two- to seven-carbon carboxylic acids, which are buffered with calcium carbonate or ammonium bicarbonate. The fermentation broth is clarified, concentrated, and dried to produce carboxylate salts, a "biocrude" that can be chemically converted to chemicals and fuels.

Acid fermentation is a key step in the MixAlco process (Figure 1-3) because it

dominates the capital costs, and determines the overall rates and yields. Mixed-culture acid fermentation is ideal for a biorefinery for the following reasons: (1) no enzyme addition, (2) no genetically modified microorganisms or mono-cultures, (3) no contaminates, (4) adapts to feedstock fluctuations, and (5) low capital and operating costs. The mixed-culture acid fermentation employs similar microorganisms as biomethane fermentations, except methanogens are inhibited with iodoform (Ross, 1998). The goal of fermentation is to maximize carboxylic acid yield (product per reactant fed) and carboxylic acid concentration (not grow cells) (Holtzapple et al., 1999).

Typically, four fermentors (Figure 6-1) are used to create a countercurrent fermentation "train" (Agbogbo, 2005; Aiello-Mazzarri, 2002; Domke et al., 2004; Fu, 2007; Ross, 1998). The first fermentor is fed with the most reactive (fresh) biomass, but has the highest product carboxylic acid concentration (greatest product inhibition). The last fermentor has the most recalcitrant (digested) biomass, but has the lowest product concentration (least product inhibition). This countercurrent strategy achieves both high product concentration and high conversion.

At first glance, the fermentation system described in Figure 6-1 appears similar to use of chemostats in series (Abu-Reesh, 2004; Davis et al., 1990; Rapaport et al., 2008). However there are differences that preclude meaningful comparison (1) flow is countercurrent rather than one-directional, (2) mixed-culture of non-competitive microorganisms (not a monoculture nor competitive microorganisms), (3) complex substrate (not a single substrate; e.g., glucose), (4) cell mass and growth rate are not quantified nor of interest, and (5) fermentation is a heterogeneous reaction with solid substrate (i.e., lignocellulose).

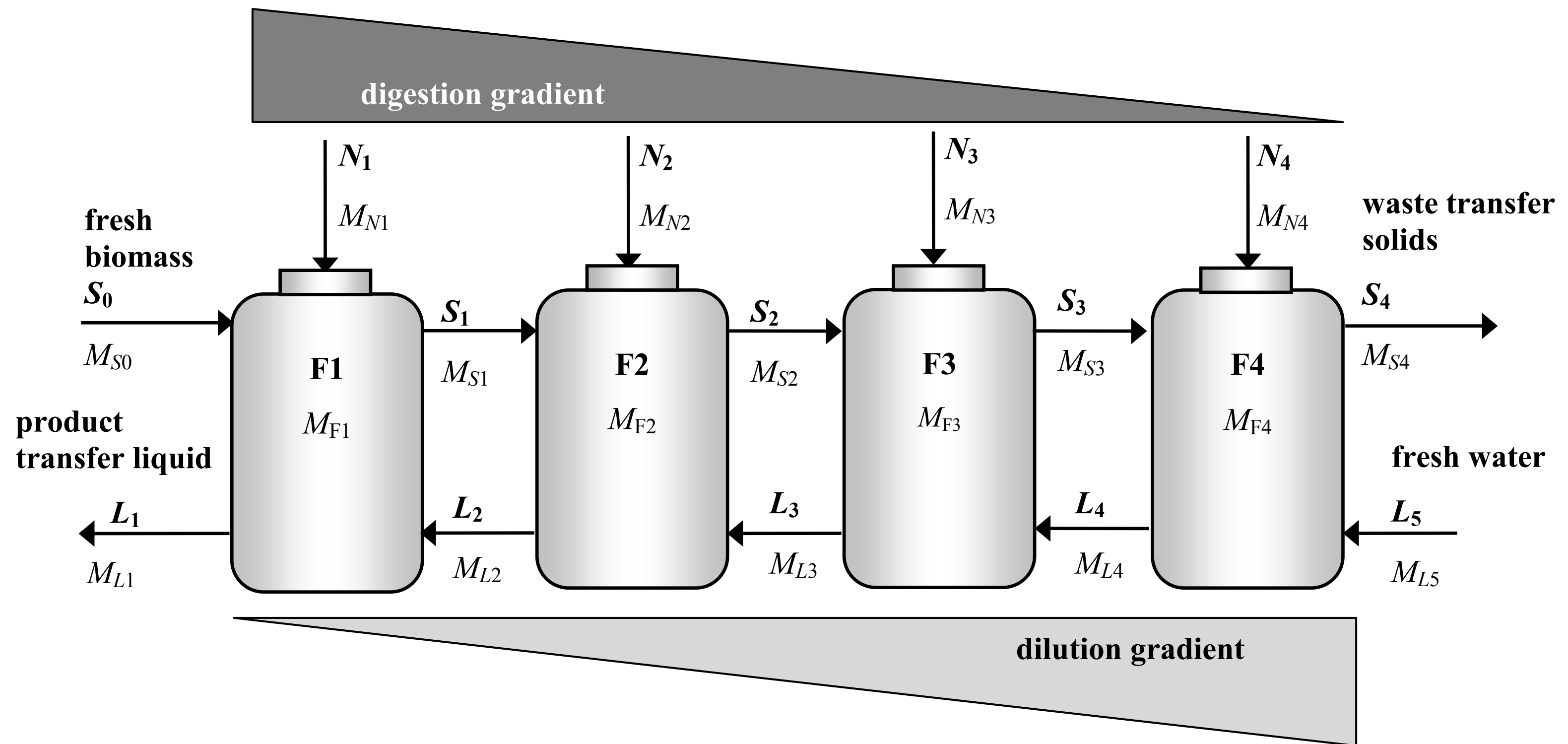


Figure 6-1. Four-stage countercurrent fermentation train with digestion and dilution gradients. S_0, L_5, and N_i are the feed carbohydrate, water, and nutrient stream flow rates (mass/time), respectively. S_i, L_i, and F_i are the transfer solids stream flowrate (mass/time), transfer liquid stream flowrate (mass/time), and total fermentation mass in Fermentor i, respectively. M_{Xi} is the moisture content of the material in the stream of fermentor.

6.1.2. Role of nitrogen transport in countercurrent staged fermentations

Carbohydrates (e.g., municipal solid waste, paper, sugarcane bagasse) and nutrients (e.g., sewage sludge, manure) ferment better when blended in an optimal ratio (Aiello-Mazzarri, 2002; Rapier, 1995). For over a decade, nutrients have been treated as though they were insoluble dry solids and were fed to Fermentor F1 along with the insoluble carbohydrates (S_0) (Agbogbo, 2005; Aiello-Mazzarri, 2002; Aiello-Mazzarri et al., 2006; Domke, 1999; Domke et al., 2004; Fu, 2007; Holtzapple et al., 1999; Loescher, 1996; Moody, 2006; Ross, 1998; Thanakoses, 2002). This practice was understandable because nutrients were typically dried for convenient laboratory use. Recently we learned that many nutrients are soluble and can leave with the product transfer liquid (L_1) (Figure 1-4) before being incorporated into microbial cells and enzymes. Further, carbon-nitrogen ratios (C/N) were not measured or controlled in these fermentation studies. Thus, it is possible that performance was restricted by nitrogen and nutrient limitations, rather than the feedstock or operating conditions.

The goal of this study was to determine the influence of different nutrient feeding strategies (i.e., amount of nutrient fed to each fermentor) on fermentation performance. It was hypothesized that (1) each nutrient feeding strategy would distribute nitrogen differently such that a unique carbon-nitrogen ratio (C/N) profile is observed, and (2) the best performance would coincide with the fermentation whose C/N profile was most near the optimal C/N ratio.

6.1.3. Carbon-nitrogen ratio (C/N)

Mixed-culture acid fermentations of lignocellulose are long (20–60 days liquid retention) and dilute (20–40 g acid/L), thus requiring large fermentors. Improving fermentation performance will significantly reduce capital costs and increase productivity. Nitrogen is required for cell replication, maintenance, metabolism, and production of enzymes (Kayhanian and Rich, 1995). Because lignocellulose hydrolysis is the rate limiting step, maintaining sufficient nitrogen concentrations/proportions is necessary to ensure that production of critical hydrolysis enzymes (e.g., cellulase) is not

restricted (Oztekin et al., 2008; Sanchez and Demain, 2002).

In biomethane fermentations, the carbon-nitrogen (C/N) ratio influences performance (Kayhanian and Rich, 1995; Liu et al., 2008). Too much nitrogen may result in ammonium toxicity (Kayhanian, 1994; Marchaim, 1992) and too little nitrogen limits cellular activity; therefore, nitrogen control is necessary for optimum performance. For countercurrent mixed-acid fermentations, no models currently exist that describe nitrogen behavior.

To quantify carbon-nitrogen ratios, the literature uses a variety of units (e.g., g total C/g total N, g starch C/g externally added N, g carbohydrate chemical oxygen demand/total Kjedahl N) (Kayhanian and Tchobanoglous, 1992; Kim et al., 2006; Oztekin et al., 2008). In this paper, *carbon-nitrogen ratio* is defined as the mass of total organic carbon minus the carbon contributed by the carboxylic acids (product) (g non-acid carbon; g C_{NA}) per mass of nitrogen (g N). With respect to acidogens, this definition of C/N ratio characterizes the relative proportion of reactant (energy) per nitrogen (nutrient). For this study, the organic acids represented 8–18% of the total carbon. If the carbon contributed by the acids is not excluded, the C/N will be overstated, which could lead to over-addition of nutrients (added cost) and sub-optimal performance.

For similar fermentations (methane and hydrogen), the literature cites a wide range of optimal C/N (10–90 g/g); 30 is the most cited optimum for producing carboxylic acids (Kim et al., 2006; Liu et al., 2008; Marchaim, 1992). Because the C/N ratio is reported in a variety of units and there are conflicting scopes of research, a new study should be done to determine the optimum C/N ratio for mixed-acid fermentations. For the purpose of discussion and reference, this paper assumes 30 g C_{NA}/g N is the optimal C/N ratio.

6.2. Materials and methods

6.2.1. Substrates

Table 6-1 lists the feedstock properties. Shredded office paper (carbohydrate source) from Texas A&M University's recycling center (College Station, Texas) and fresh (wet) chicken manure (nutrient source) from Feathercrest Farm (Bryan, Texas) were used in a 4:1 carbohydrate:nutrient ratio on a dry mass basis. Paper was selected because it is free of lignin and did not require pretreatment. No additional nutrients (bloodmeal, urea, etc.) were added. The C/N ratio of the feed was 39 ± 1 g C_{NA}/g N.

Table 6-1. Feedstock properties.

	Office paper	**Fresh chicken manure**
Moisture content, M (g H_2O/g wet sample)	0.051 ± 0.03	0.660 ± 0.03
Ash content, I (g ash/g dry sample)	0.130 ± 0.06	0.592 ± 0.09
Carbon content, C (g C/100 g wet sample)	36.3 ± 0.8	6.91 ± 0.7
Nitrogen content, N (g N/100 g wet sample)	0.25 ± 0.07	1.10 ± 0.2
Carbon-nitrogen ratio (g C_{NA}/g N)	138.3 ± 43	6.3 ± 0.7

Error values represent one standard deviation

Fermentation media

Deoxygenated water was prepared by boiling deionized water to liberate dissolved oxygen gas. After cooling to room temperature in a covered vessel, 0.275 g sodium sulfide and 0.275 g cysteine (reducing agents) were added per liter of water.

Methanogen inhibitor

Each day, a small amount (80 μL) of methanogen inhibitor (20 g iodoform/L 200-proof ethanol) was added to each fermentor bottle (Ross, 1998).

Inoculum

The inoculum was obtained from the MixAlco Pilot Plant (College Station, TX),

which was originally inoculated with marine microorganisms from Galveston, TX. The mixed cultures were dominated by *Clostridia* species.

6.2.2. Analytical methods

Acid concentration

Ultra-centrifuged (15,000 rpm) fermentation liquid was mixed with equal parts of internal standard (1.162 g/L 4-methyl-*n*-valeric acid) and 3-M H_3PO_4. The H_3PO_4 ensures that carboxylate salts are converted to carboxylic acid prior to analysis. The carboxylic acid concentration was measured using an Agilent 6890 Series Gas Chromatograph (GC) system equipped with a flame ionization detector (FID) and an Agilent 7683 automatic liquid sampler. A 30-m fused-silica capillary column (J&W Scientific Model # 123-3232) was used. The column head pressure was maintained at 2 atm (absolute). After each sample injection, the GC temperature program raised the temperature from 40 °C to 200 °C at 20 °C/min. The temperature was subsequently held at 200 °C for 2 min, with a total run time per sample of 11 min. Helium was the carrier gas. The calibration standard was volatile acid mix (Matreya, LLC, Cat No. 1075).

Gas composition

Each fermentor was vented daily to relieve pressure and prevent rupture. The gas volume was measured by liquid displacement using an inverted graduated glass cylinder filled with an aqueous solution of 300 g $CaCl_2$/L to prevent microbial growth and carbon dioxide absorption (Agbogbo, 2005; Aiello-Mazzarri, 2002; Chan, 2002; Domke, 1999; Fu, 2007). To monitor methane, 5-mL gas samples were taken through the fermentor septum then analyzed by the Agilent 6890 Series Chromatograph with a thermal conductivity detector (TCD). Samples were injected manually. A 4.6-m stainless steel packed column with 2.1-mm ID (60/80 Carboxen 100, Supelco 1-2390) was used. The inlet temperature was 230 °C, the detector temperature was 200 °C, and the oven temperature was 200 °C. The total run time was 10 min. Helium was the carrier gas.

Carbon and nitrogen contents

The C/N ratio was characterized using total carbon and total nitrogen contents, both of which were measured in a single test using an Elementor Variomax CN. Total organic carbon is preferred in the C/N ratio, but because 99% of the total carbon fed was organic carbon, the added cost of distinguishing the two was not justified. The C/N ratio was used to compare trends among the different nutrient feeding strategies. Because each train was fed the same feedstocks, these trends are similar, regardless of whether total carbon or total organic carbon was used. No external buffer, such as calcium carbonate, was added because minerals in the feed self-regulated the pH between 5.5 and 6.5. Total carbon and total nitrogen contents (g/100 g) were determined by Texas A&M University Soil, Water, and Forage Testing Lab (College Station, TX). The nitrogen compounds were undefined; only the total nitrogen content was of interest.

Moisture and ash contents

Moisture contents (M_{Xi}) and ash contents (I_{Xi}) were measured in series. First the sample was dried in a 105 ºC forced-convection oven (>12 h) and then ashed in a 550 ºC furnace (>3 h). Before drying, 3 g $Ca(OH)_2$/100 g sample was added to ensure all volatile acids were converted to salts and retained during drying. This practice disproportionately overstates the ash content; thus, exit-stream ash data were unreliable. To overcome this problem, the consumption of non-acid volatile solids (NAVS) was determined using the inert-ash approach (Section 6.2.3).

Definition of terms

Figure 6-2 shows the conversion of biomass using mixed-acid fermentation. The feed consists of initial volatile solids $VS_{initial}$, which are composed of undigested VS, other VS (e.g., “scum”, energy storage compounds, proteins), cells, and carboxylic acids (typically in the nutrient source). Enzymes produced by the mixed-culture of acid-forming microorganisms hydrolyze polymers (e.g., cellulose, hemicellulose) into sugars, which are subsequently fermented into carboxylic acids, gases, cells, and other VS. Ash is assumed to be inert.

Previous works defined *conversion* as volatile solids (VS) digested per VS fed (Agbogbo, 2005; Aiello-Mazzarri, 2002; Aiello-Mazzarri et al., 2006; Blasig, 1991; Blasig et al., 1992; Chan, 2002; Coleman, 2007; Domke, 1999; Domke et al., 2004; Fu, 2007; Moody, 2006; Ross, 1998; Thanakoses, 2002). Based on this definition, cellular components (e.g., cells, extracellular proteins, energy-storage compounds, and "scum") are considered products. To accurately satisfy this definition, these non-acid digestion products must be measured and accounted – a very difficult task. The analytical procedure presented by the above-cited authors did not separately measure cellular components, so their results were inconsistent with their definition. This paper presents a simpler definition of conversion that can be applied to any fermentation.

Mixed-acid fermentations digest a wide variety of biological components, including cellulose, hemicelluloses, starch, free sugars, pectin, proteins, fats, and dead cells. Some studies state that anaerobic cultures can ferment lignin to some extent (Kondo et al., 1999; Susmel and Stefanon, 1993). As a consequence, all the VS – except for carboxylic acids – represent potential reactants. Based on this observation *reactants* are defined as non-acid volatile solids (NAVS). Thus, undigested biomass, cells, extracellular proteins, energy-storage compounds, "scum," and all other NAVS are considered reactants. This definition simplifies a complicated reaction system into four quantifiable and industrially meaningful terms: water (solvent), ash (inert), acid (product), and NAVS (reactant). Figure 6-2 illustrates the conversion of biomass in fermentation. The water of hydrolysis maybe estimated by assuming the biomass is predominately cellulose (monomer weight of 162 g/mol). When a cellulose monomer is hydrolyzed, it gains one mole of water.

$$\text{water of hydrolysis (g)} = \text{NAVS}_{\text{consumed}}\ \text{(g)} \times \frac{18}{162} \tag{6-1}$$

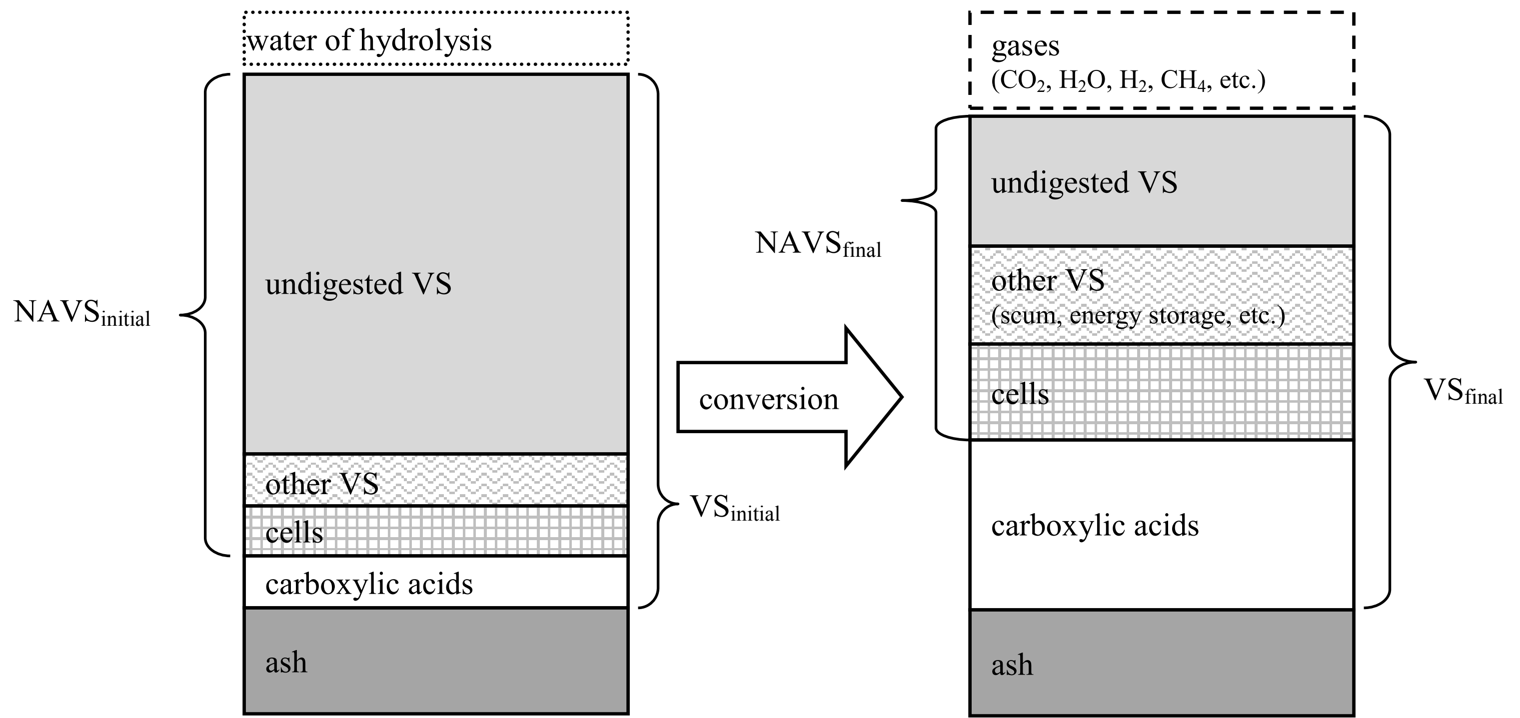


Figure 6-2. The conversion of biomass.

Previous works defined *yield* as the mass of total acids produced per mass of volatile solids (VS) fed (Agbogbo, 2005; Aiello-Mazzarri, 2002; Aiello-Mazzarri et al., 2006; Blasig, 1991; Blasig et al., 1992; Domke, 1999; Fu, 2007; Loescher, 1996; Moody, 2006; Rapier, 1995; Ross, 1998). The researchers assumed the sum of carboxylic acids in the product liquid and the waste transfer solids were entirely produced by the fermentation system. Analysis of the chicken manure used in this experiment shows that the feed contains a significant concentration of organic acids (~45 g/L_{liq}), which contributes 0.022 g acid/g NAVS fed. Without accounting for the carboxylic acids in the feed, selectivity is overstated and yield is ill-defined. To provide clarity and context, this section introduces four definitions of yield (Equations 10–13) with respect to different points in the fermentation system: feed, exit streams, microbial culture, and product transfer liquid stream. The *exit yield* is identical to the yield calculated in previous works.

Referring to the labels defined in Figure 6-1, the following terms are used in this paper:

$$\mathrm{NAVS_{feed}}\ (\mathrm{g}) \equiv \text{sum of NAVS in } S_0, N_1, N_2, N_3, N_4, \text{ and } L_5 \tag{6-2}$$

$$\mathrm{NAVS_{exit}}\ (\mathrm{g}) \equiv \text{sum of NAVS in } S_4 \text{ and } L_1 \tag{6-3}$$

$$\mathrm{NAVS_{consumed}}\ (\mathrm{g}) \equiv \mathrm{NAVS_{feed}} - \mathrm{NAVS_{exit}} \tag{6-4}$$

$$A_{\mathrm{feed}}\ (\mathrm{g}) \equiv \text{sum of carboxylic acid in } S_0, N_1, N_2, N_3, N_4, \text{ and } L_5 \tag{6-5}$$

$$A_{\mathrm{exit}}\ (\mathrm{g}) \equiv \text{sum of carboxylic acid in } S_4, L_1, \text{ and any liquid samples removed from F2–F4} \tag{6-6}$$

$$A_{\mathrm{produced}}\ (\mathrm{g}) \equiv A_{\mathrm{exit}} - A_{\mathrm{feed}} \tag{6-7}$$

$$A_{L_1}\ (\mathrm{g}) \equiv \text{total carboxylic acid in } L_1 \tag{6-8}$$

$$\text{conversion} \equiv C \equiv \frac{\text{NAVS}_{\text{consumed}}}{\text{NAVS}_{\text{feed}}} \tag{6-9}$$

$$\text{yield}_{\text{feed}} \equiv Y_F \equiv \frac{A_{\text{feed}}}{\text{NAVS}_{\text{feed}}} \tag{6-10}$$

$$\text{yield}_{\text{exit}} \equiv Y_E \equiv \frac{A_{\text{exit}}}{\text{NAVS}_{\text{feed}}} = Y_F + Y_C \tag{6-11}$$

$$\text{yield}_{\text{culture}} \equiv Y_C \equiv Y_E - Y_F \equiv \frac{A_{\text{produced}}}{\text{NAVS}_{\text{feed}}} = C \cdot \sigma \tag{6-12}$$

$$\text{yield}_{\text{process}} \equiv Y_P \equiv \frac{A_{L_1}}{\text{NAVS}_{\text{feed}}} \tag{6-13}$$

$$\text{total acid selectivity} \equiv \sigma \equiv \frac{Y_C}{C} \tag{6-14}$$

$$\text{total acid productivity (train)} \equiv P \equiv \frac{A_{\text{produced}}}{\text{TLV} \times \text{time}} \tag{6-15}$$

where TLV (total liquid volume) is defined in Equation 6-24.

Exit yield includes the carboxylic acid removed in product liquid (L_1), waste transfer solids (S_4), and liquid samples taken from Fermentors F2–F4. The cumulative amount of carboxylic acid in the samples is less than 1% of the total acid produced. NAVS removed when sampling liquids and bulk material (unfiltered solids and liquid) was not quantified. The amount of NAVS in the sampling liquid is negligible and bulk samples (~5 g) were taken only two times for C/N ratio analysis.

Acetic acid equivalents (aceq) equate the reducing potential of a carboxylic acid mixture to an energy-equivalent mass of acetic acid (Datta, 1981). Concentrations are converted to acetic acid equivalents using the following equation:

$$
\begin{aligned}
\alpha\ (\text{mol/L}) \quad = \quad & \text{acetic (mol/L)} \\
& + 1.75 \cdot \text{propionic (mol/L)} \\
& + 2.50 \cdot \text{butyric (mol/L)} \\
& + 3.25 \cdot \text{valeric (mol/L)} \\
& + 4.0 \cdot \text{caprioc (mol/L)} \\
& + 4.75 \cdot \text{heptanoic (mol/L)} \qquad \text{(6-16)}
\end{aligned}
$$

On a mass basis, acetic acid equivalents are defined as

$$
\text{aceq}\ \left(\frac{\text{g}}{\text{L}}\right) = 60.05\ \left(\frac{\text{g}}{\text{mol}}\right) \cdot \alpha\ \left(\frac{\text{mol}}{\text{L}}\right) \qquad \text{(6-17)}
$$

6.2.3. Measuring performance

Slope method

During the steady-state period, the flowrate (amount/day) of acid, ash, NAVS, water, and gas were determined. The fermentations trains were semi-continuous with material transfers performed three times per week. To determine the flowrate of a component, the moving cumulative sum of that component was plotted with time. The component flowrate (amount/day) was determined from the slope of the line. All performance variables were calculated from component flowrates determined by the Slope method (see Section 5 for more details).

Determination of NAVS consumed

The $\text{NAVS}_{\text{consumed}}$ is the difference between the NAVS in the inlet and exit streams. This quantity can be determined by two approaches: (1) direct measurement and (2) inert ash.

Direct measurement – The NAVS component flowrate in inlet and outlet streams (S_0, L_5, N_1, N_2, N_3, N_4, S_4, L_1) are measured directly using Equation 6-18 and the slope method.

$$\text{NAVS}_{X_i} = X_i \left(\left(1 - M_{X_i}\right)\left(1 - I_{X_i}\right) - \frac{[A]_{X_i} M_{X_i}}{\rho_{\text{water}}} \left(\frac{1\ \text{L}}{1000\ \text{mL}}\right) \right) \qquad (6\text{-}18)$$

where

X_i = total transferred mass of Stream X_i (g)

M_{X_i}= moisture content of Stream X_i (g moisture/g wet sample)

I_{X_i} = ash content of Stream X_i (g ash/g dry sample)

$[A]_{X_i}$ = total carboxylic acid concentration (g/L_{Liq}) of Stream X_i

ρ_{water} = density of water (1 g/mL)

The total inlet $NAVS_{feed}$ flowrate minus the $NAVS_{exit}$ flowrate equals the $NAVS_{consumed}$ rate.

Inert-ash approach – Assuming ash is inert, the ash flowrates in and out are equal. Based on this assumption, the difference between the dry material in the inlet and outlet streams results from the change in VS, not a change in ash. The $NAVS_{consumed}$ rate (g $NAVS_{consumed}$/d) may be determined by Equation 6-19.

$$\begin{aligned} \text{NAVS}_{\text{consumed}}\ \text{rate} &= \text{NAVS}_{\text{feed}}\ \text{rate} - \text{NAVS}_{\text{exit}}\ \text{rate} \\ &= (\Sigma\text{dry solids}_{\text{in}} - \Sigma\text{ash}_{\text{Iin}} - \Sigma\text{acid}_{\text{in}}) - (\Sigma\text{dry solids}_{\text{out}} - \Sigma\text{ash}_{\text{in}} - \Sigma\text{acid}_{\text{out}}) \\ &= (\Sigma\text{dry solids}_{\text{in}} - \Sigma\text{acid}_{\text{in}}) - (\Sigma\text{dry solids}_{\text{out}} - \Sigma\text{acid}_{\text{out}}) \qquad (6\text{-}19) \end{aligned}$$

where:

$$\text{dry solids in stream } X_i\ (\text{g}) = X_i\,(1 - M_{Xi}) \qquad (6\text{-}20)$$

$$\text{acid in stream } X_i \text{ (g)} = A_{X_i} = \frac{X_i\,[A]_{X_i}\,M_{X_i}}{\rho_{water}} \left(\frac{1\text{ L}}{1000\text{ mL}}\right) \quad (6\text{-}21)$$

The inert-ash approach was used to calculate conversion because it is independent of ash content measurements (which were inaccurate for this experiment). Ideally, both methods would give the same result.

6.2.4. Operating parameters

Moisture retention time (MRT) (Equation 6-22) quantifies the average time for liquid to travel through the system. MRT influences the product concentration ($[A]_{L1}$). Longer residence times allow for higher product concentrations.

$$\text{MRT} = \frac{\text{TLV}}{\text{Q}} \quad (6\text{-}22)$$

TLV is the total liquid volume expressed as

$$\text{TLV} = \sum_i \left(\frac{K_{Fi} M_{Fi}}{\rho_{water}} \left(\frac{1\text{ L}}{1000\text{ mL}}\right) + L_{Fi} \right) \quad (6\text{-}23)$$

where,

L_5, S_0, and N_i are rates determined by the Slope method (g/d)

K_{Fi} = the average mass of wet solid cake in Fermentor i (g),

L_{Fi} = the average volume of free liquid in Fermentor i (L).

where the total inlet flowrate Q is determined using Equation 6-23 and the Slope method:

$$\text{Q} = \left(L_5 M_{L5} + S_0 M_{S_0} + \sum_i N_i M_{N_i} \right) \frac{1}{\rho_W} \left(\frac{1\text{ L}}{1000\text{ mL}}\right) \text{ (L/d)} \quad (6\text{-}24)$$

Previous works define the liquid flowrate as the product liquid exit F1 (L_1) and ignoring the liquid that exits with the waste solids (S_4). Compared to LRT (See Section 4.4.5 for more details.), MRT as defined by Equation 6-24 more accurately quantifies the average time liquid is retained in the staged fermentation.

Volatile solids loading rate (VSLR) quantifies the reactant feed rate relative to the total liquid volume and is defined as

$$\text{VSLR} = \frac{\text{NAVS}_{\text{feed}}\text{ rate}}{\text{TLV}} \tag{6-25}$$

VSLR is inversely related to conversion and yield (Agbogbo, 2005; Domke, 1999; Thanakoses, 2002). As VSLR increases, NAVS have less time to digest, which lowers conversion and yield.

The NAVS concentration ($\text{SC}_{\text{F}i}$) (Equation 6-26) is defined as the ratio of reactant in Fermentor Fi (NAVS_{Fi}) to the liquid volume in Fermentor Fi (LV_{Fi})

$$\text{SC}_{Fi} \equiv \text{NAVS}_{Fi}/\text{LV}_{Fi} \tag{6-26}$$

Acid concentration is directly proportional to SC (Lee, 1993).

6.2.5. *Steady-state strategy*

Transfer solids physically appear solid, but have moisture contents of 0.70–0.85 g moisture/g total with all moisture fully absorbed in the biomass. Transfer liquids physically appear fluid, but may have 1–5% suspended solids.

For a countercurrent staged fermentation (Figure 6-1), there are eight degrees of freedom. The following six operating parameters are completely independent: (1) temperature, (2) pressure, (3) pH, (4) transfer frequency (transfer/time), (5) solids retained in each fermentor (total mass), and (6) liquid retained in each fermentor (total

mass or volume). The remaining two operating parameters are selected from the following: reactant feed rate (S_0), waste transfer solid rate (S_4) (amount/transfer), liquid feed rate (L_5), and product transfer liquid rate (L_1) (amount/transfer). For laboratory fermentations, the reactant feed rate (S_0) and liquid feed rate (L_5) are typically held constant. For logistical reasons, large-scale operations may have to control the reactant feed rate (S_0) and product transfer liquid rate (L_1). pH is typically controlled via a buffer. Pressure is not controlled but fluctuates between atmospheric and the natural pressure built up in the fermentation because of gas production.

Table 6-2 summarizes the operating parameters of the five trains described in this paper. The normalized operating parameters (NOP) are calculated from the controllable operating parameters. NOP *cannot be directly controlled* because they depend on the fermentation moisture and ash contents, which are dictated by fermentation performance.

Before a transfer, each fermentor and its contents were centrifuged at 4000 rpm. The liquid layer was decanted into a graduated cylinder and measured. The bottle and remaining solid cake were weighed (B_i), where i equals the fermentor number. For F1, the amount of transfer solids fed (S_0) was constant. For subsequent fermentors (Fi), the transfer solids fed was equal to the transfer solids removed (S_{i-1}) from the previous fermentor plus the nutrient fed to that fermentor (N_i). The transfer solids retained in each fermentor were controlled by a solids-retained-plus-bottle-weight set point (W_i). The mass of transfer solids removed (S_i) was determined by a simple material balance ($S_i = B_i + S_{i-1} + N_i - W_i$). For each train, the solids-retained-plus-bottle-weight set point for F1 was 200 g and 300 g for F2 to F4. The set point for F1 was lower because fresh paper absorbed free transfer liquid added to F1. All decanted transfer liquid was transferred to the previous fermentor, as shown in Figure 1-4.

Table 6-2. Operating parameters for nutrient contacting fermentations.

	Fermentation Train	1	2	3	4	P	AVG
Controllable	Temperature (˚C)	40	40	40	40	40	40
	Frequency (T)*	3 per week; every 56 h					
	$NAVS_{feed}$ rate (paper & manure) (g VS/T)*	30.4	30.4	30.4	30.4	30.4	30.4
	Liquid feed rate (L_5) (mL/T)*	300	300	300	300	300	300
	Solid-cake-plus-bottle-weight set point, F1 (g)	200	200	200	200	200	200
	Solid-cake-plus-bottle-weight set point, F2–F4 (g)	300	300	300	300	300	300
	Centrifuge liquid retained in F1–F4 (mL)	0	0	0	0	0	0
	Methane inhibitor (μL/T)*	80	80	80	80	80	80
Normalized	VSLR (g NAVS/(L_{liq}·d))	7.5	6.7	6.8	7.1	7.0	7.0
	MRT (d)	13.6	15.2	15.0	14.2	14.6	14.5
	Avg. solid concentration (g NAVS/L_{liq})	57	49	48	55	53	52
	TLV (L)	1.75	1.96	1.93	1.83	1.87	1.87

*T = transfer (~56 h)

6.2.6. Statistical methods

To compare steady-state acid data, the two-tailed heteroscedastic student t-test (“TTEST” function in Microsoft Excel 2007) with a confidence level of 5% was used to calculate *p*-values. Unless otherwise stated, error bars represent a 95% confidence interval (two standard deviations). Sum-of-squared-errors techniques were used to determine the error of calculated values.

6.3. Results and discussion

6.3.1. Experiment overview

Five four-bottle trains (Figure 6-3) were run with identical operating parameters (Table 6-2), each with a different nutrient contacting pattern. Many variables influence fermentation performance (SC, VSLR, MRT, substrates, solid-liquid separation efficiency, number of stages, etc.). The interaction of operating parameters and nutrient addition strategies is complex, so these results must be carefully interpreted and applied in context with operating parameters used in this study.

6.3.2. C/N ratio and productivity profiles

Figure 6-4 shows the C/N ratio profile produced by each nutrient loading pattern. Overall C/N ratio is defined as the sum of non-acid carbon (g C_{NA}) in all fermentors divided by the sum of total nitrogen (g N) in all fermentors. Train 1 produced the most even C/N profile with ratios slightly increasing in successive stages. Train 2 had a high C/N ratio (90 g C_{NA}/g N) in F1, but F2–F4 had C/N ratios very close to the optimum of 30 g C_{NA}/g N. Train 4 had the most uneven C/N profile. Trains 3, 4, and P had overall C/N ratios greater than the feed (39 ± 1 g C_{NA}/g N), indicating distribution inefficiencies and/or gaseous nitrogen loss. Each train had one or more bottles with a C/N ratio above 30 g C_{NA}/g N indicating nitrogen limitations; thus, no train was fully optimized.

Total acid productivity is defined as the acid *produced* per liquid volume per day; thus, the acid contributed by the nutrient (chicken manure) is *not* included. Figure 6-5 shows the productivity profile of each train. Overall productivities are weighted averages with the total liquid volume of each bottle.

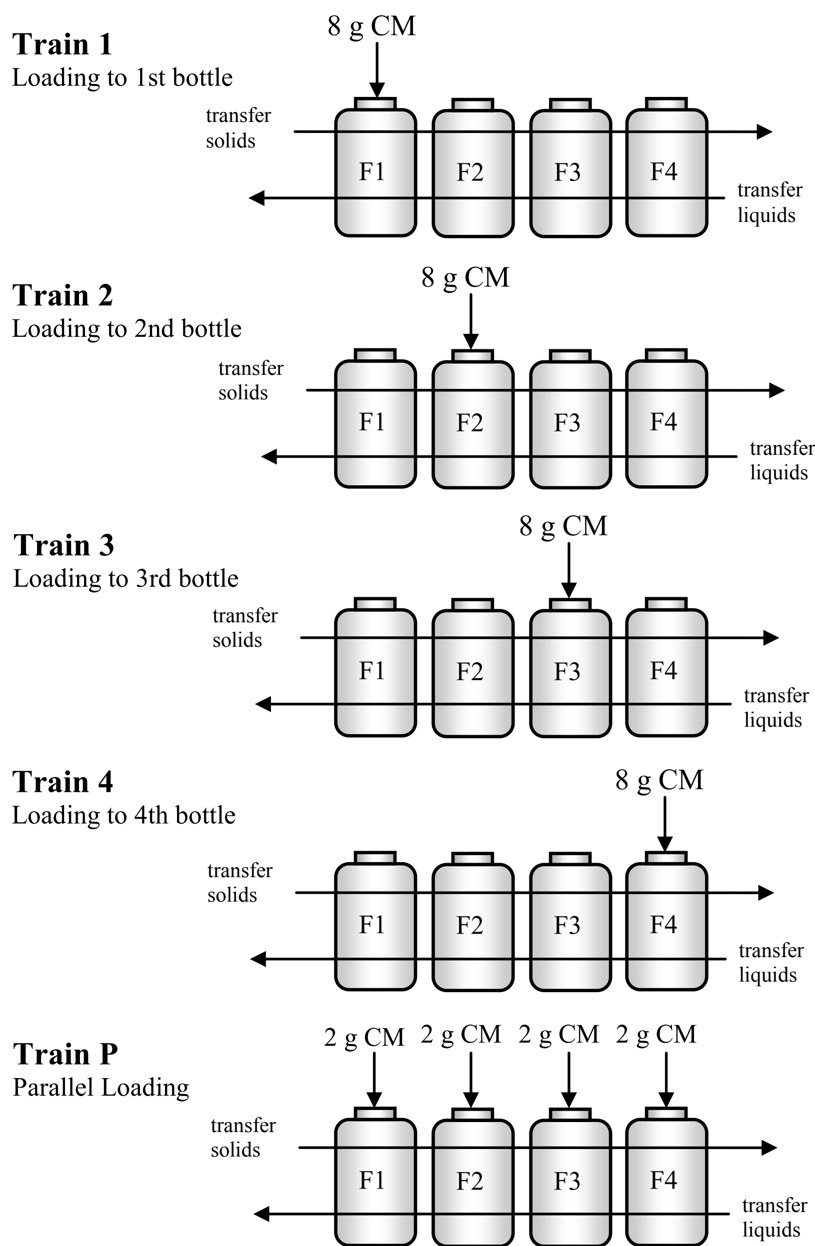
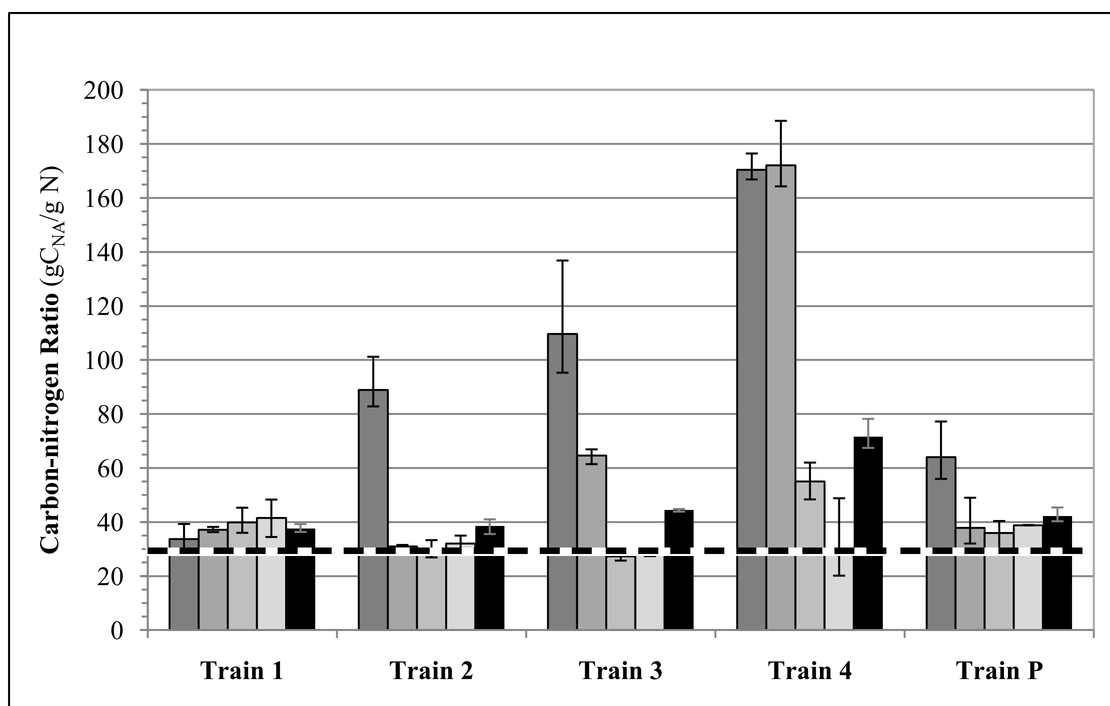
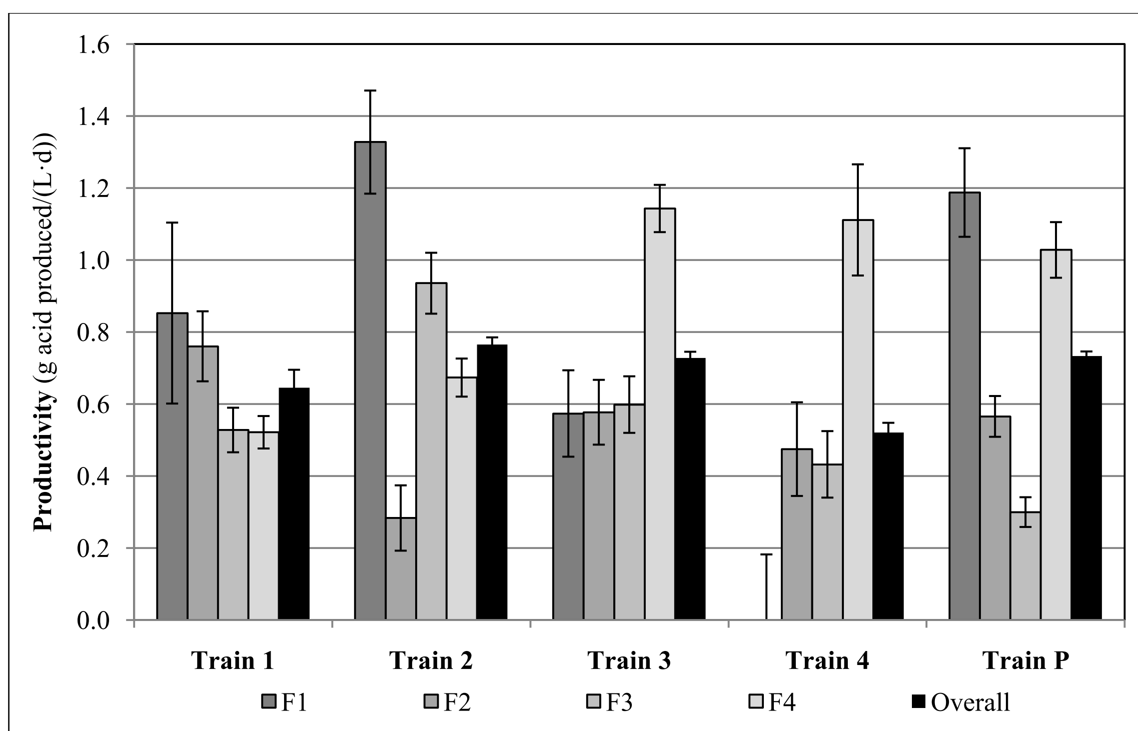


Figure 6-3. The nutrient loading pattern for Trains 1, 2, 3, 4, and P with the amount of wet chicken manure (CM; on a dry basis) added to each fermentor.



	F1		F2		F3		F4		Overall	
Train 1	33.7	29.5 39.3	37.1	36.3 38.2	39.9	36.0 45.3	41.5	48.3 34.5	37.6	36.3 39.2
Train 2	88.9	82.8 101.2	30.9	31.5 30.1	30.1	26.9 33.3	32.0	29.2 35.0	38.5	41.0 35.5
Train 3	109.6	95.3 136.8	64.6	66.9 61.4	27.3	28.3 25.7	27.9	28.4 27.4	44.4	44.8 43.9
Train 4	170.5	166.8 176.5	172.1	164.3 188.5	55.0	48.4 62.0	29.1	20.1 48.8	71.5	67.4 78.2
Train P	64.1	56.0 77.2	37.9	32.0 49.0	36.0	40.4 29.7	38.8	38.8 38.9	42.2	40.3 45.4

Figure 6-4. Carbon-nitrogen ratio profiles for each train. Carbon contributed by organic acid was excluded. Two profile samples were taken (Days 138 and 162). The data table lists the average (large font) and range (small font) of C/N ratios measured. The error bars represent the range. The average C/N ratio was determined by dividing the average non-acid carbon content by the average nitrogen content. The dotted line references the assumed optimum carbon-nitrogen ratio of 30 g C_{NA}/g N.



	F1	F2	F3	F4	Overall	
Train 1	0.85± 0.25	0.76± 0.10	0.53± 0.06	0.52± 0.05	0.64± 0.05	vs.Train 1
Train 2	1.33± 0.14	0.28± 0.09	0.94± 0.08	0.67± 0.05	0.77± 0.02	18.7%
Train 3	0.57± 0.12	0.58± 0.09	0.60± 0.08	1.14± 0.07	0.73± 0.02	12.8%
Train 4	0.00± 0.18	0.47± 0.13	0.43± 0.09	1.11± 0.15	0.52± 0.03	–19.3%
Train P	1.19± 0.12	0.57± 0.06	0.30± 0.04	1.03± 0.08	0.73± 0.01	13.6%
AVG	0.79	0.53	0.56	0.90	0.68	

Figure 6-5. Productivity profiles for each train. Overall values represent the composite productivity of the train.

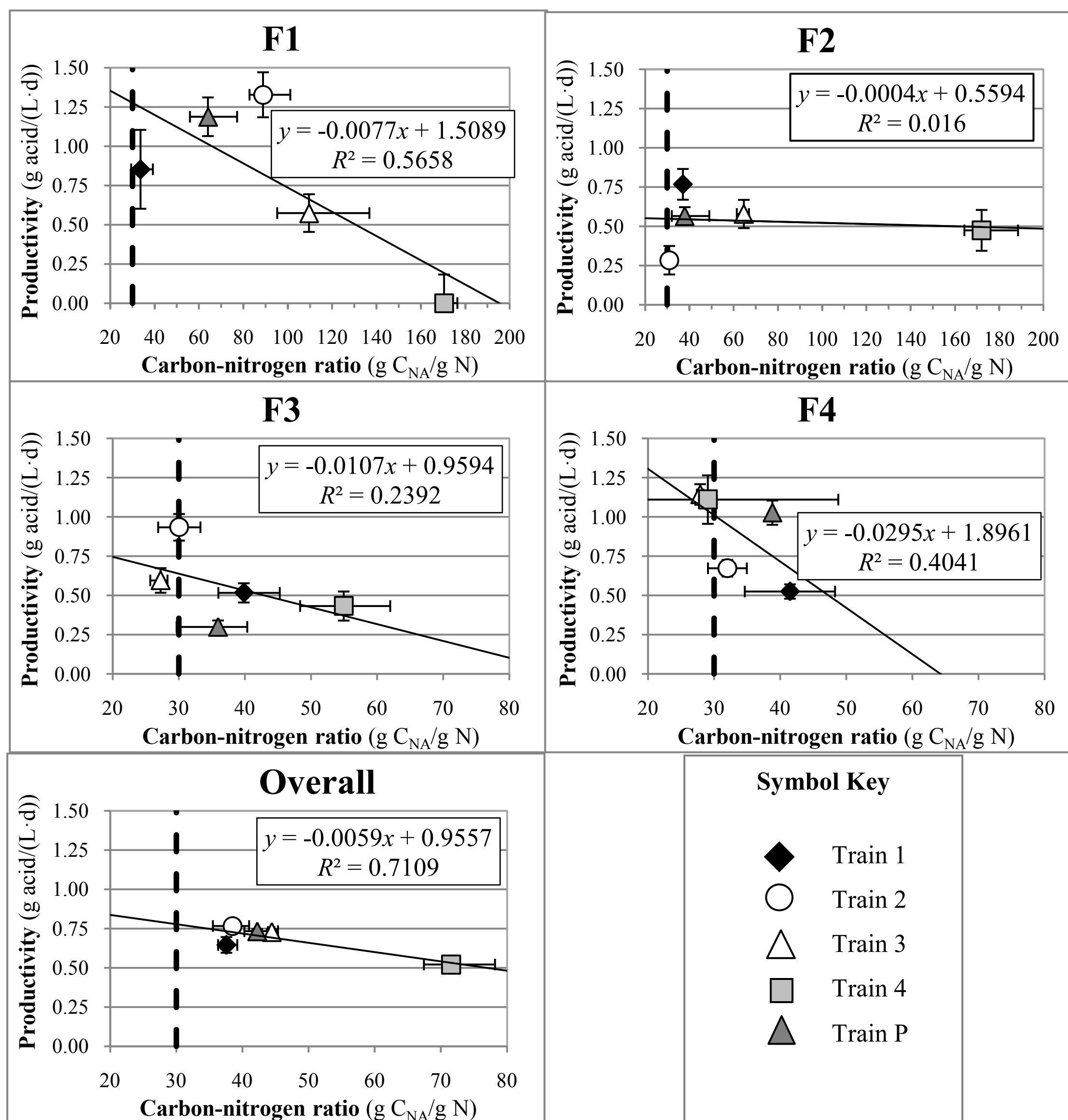


Figure 6-6. Correlation between productivity and C/N ratio for individual fermentors and train. The productivity error bars represent a 95% confidence interval (2 standard deviations). The C/N ratio error bars represent the range. The dotted line references the assumed optimum C/N ratio of 30 g C_{NA}/g N.

Although Trains 1 and 2 have virtually identical overall C/N ratios (37.6 and 38.5 g C_{NA}/g N, respectively), Train 2 had a much higher overall productivity (0.77 vs. 0.64 g acid produced/ (L_{Liq}·d)). This resulted because Train 2 distributed nitrogen such that a greater percentage of its fermentation mass was closer to the optimum C/N ratio than Train 1. In contrast, Train P had a higher C/N profile (42.2 g C_{NA}/g N, overall) and a higher productivity (0.73 g acid produced/(L_{liq}·d)) than Train 1. This indicates the importance of non-nitrogen nutritional factors (e.g., phosphorus, minerals, etc.) and/or "freshness" of nutrients. F1 and F2 of Train 4 had similar C/N ratios around 170 g C_{NA}/g N, and similar steady-state acid concentrations around 13.8 g acid/L_{liq}. Despite receiving fresh paper, F1 of Train 4 had a productivity of zero, which indicates severe nitrogen and non-nitrogen nutrient limitations.

When comparing individual fermentors from each train, those that received the full amount of fresh nutrients *did not* have the highest productivity. A possible explanation for this phenomena is the carboxylic acid content (not the nutrients) of the chicken manure caused product inhibition that reduced productivity.

Figure 6-6 shows that total acid productivity depends on C/N ratio and increases as the C/N ratio approaches the optimum. The slope of the linear trend line indicates how sensitive a fermentor is to C/N ratio. F2 had the flattest slope indicating it was the least sensitive, whereas F4 had the steepest slope indicating the greatest sensitivity. This trend is understandable considering F4 contains the most recalcitrant biomass; thus, nitrogen is critical for digestion.

Further improvements in performance can be realized if optimal C/N ratios can be maintained in each fermentor. Using Figure 6-6 to predict the productivity of each fermentor at a C/N of 30 g C_{NA}/g N suggests that overall productivities ranging from 0.83 to 0.99 g acid/(L·d) could be obtained (VSLR = 7 g NAVS/(L_{liq}·d) and MRT = 15 d). If obtained, these productivities translate into culture yield improvements of 67–99% (0.138–0.165 g acid produced/g NAVS fed) verses Train 1.

6.3.3. Acid concentration

Figure 6-7 shows the total acid concentration and aceq concentration in the product transfer liquid (L_1). Initially, the operating parameters did not produce transfer liquid from F1 because the paper loading rate (S_0) was too high relative to the water throughput (L_5); there was no free liquid because all liquid was absorbed in the fresh paper. To correct this, the solids-retained-plus-bottle-weight setpoint for F1 (W_1) of each train was decreased from 300 to 200 g (Day 20) and the water fed per transfer was increased from 175 to 300 mL per (Day 27). Thus, the noise/peak prior to steady state resulted from very high initial solids concentrations.

To compare acid concentrations with different compositions of different molecular weight volatile fatty acids, acid concentrations are converted to acetic acid equivalents (aceq). Calculation of performance variables on an aceq basis allows comparison of the energy content of the carboxylic acid products. Train 2 had the highest average steady-state acid concentration (21.3 g/L) with Trains 1, 3, 4, and P having concentrations of 20.9, 18.7, 13.9, and 20.2 g/L, respectively. The student t-test showed that Train 2 was not significantly different than Train 1 (p-value = 0.162). Train 1 had the highest average steady-state aceq concentration (28.0 g/L) with Trains 2, 3, 4, and P having concentrations of 27.2, 25.6, 18.2, and 26.1 g/L, respectively. Trains 1, 2, 3, and P had similar total acid and aceq product concentrations indicating that the nutrient loading pattern did not significantly affect product concentration. The ratio of aceq concentration to total acid concentration for Trains 1, 2, 3, 4, and P is 1.33, 1.28, 1.37, 1.31, and 1.29, respectively. Train 3 has a higher ratio than the other four trains indicating it produced more high-molecular-weight acids.

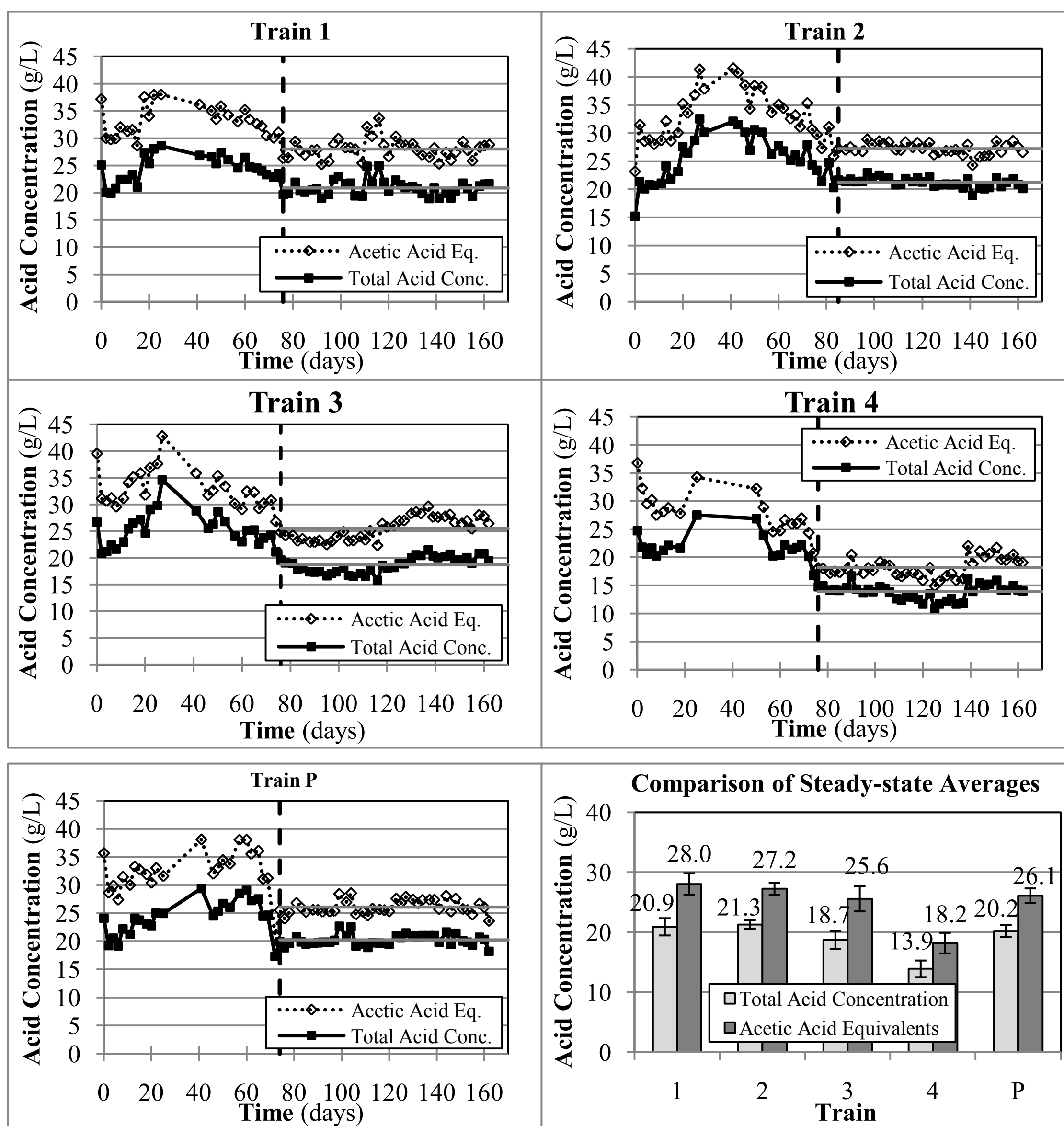
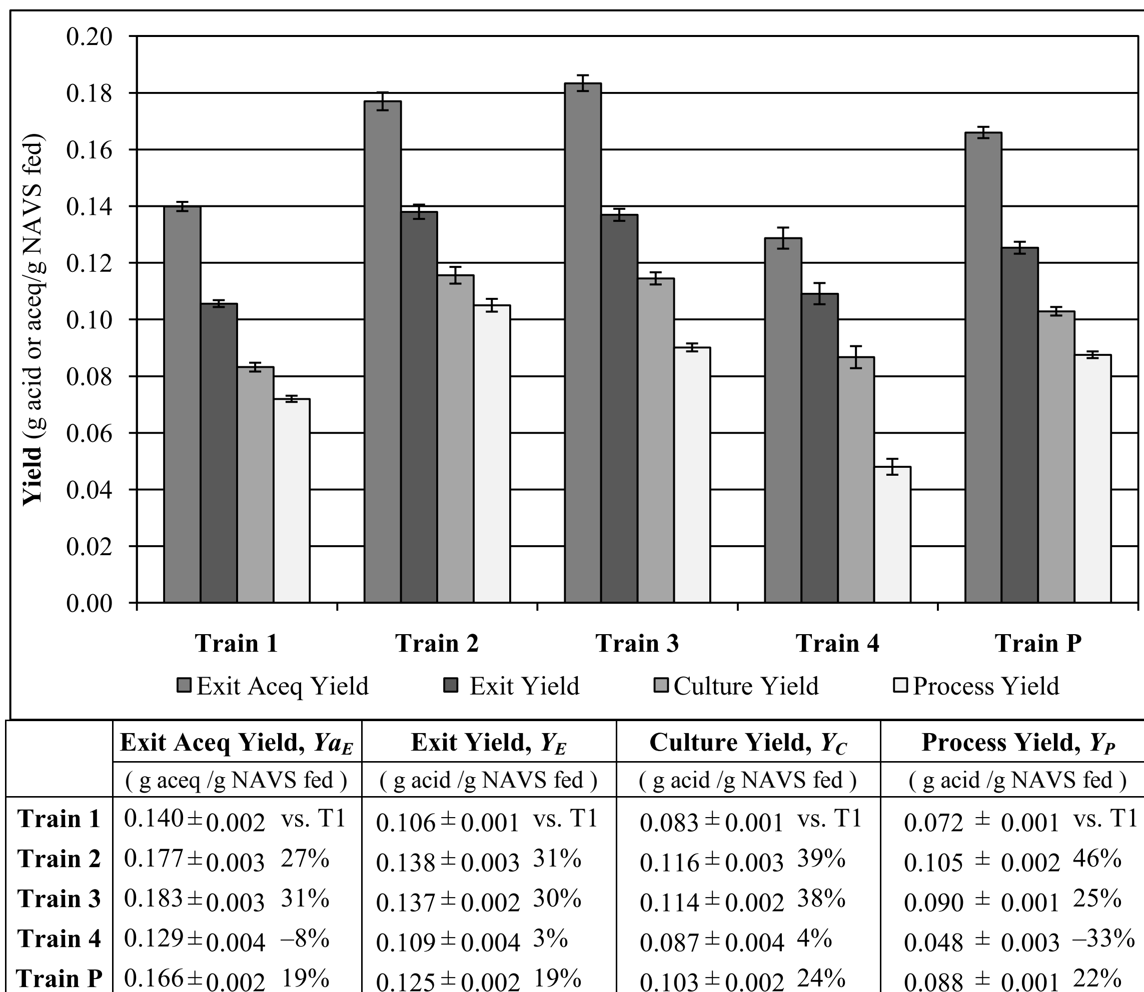


Figure 6-7. Total acid concentration and acetic acid equivalence concentration plots. The steady-state region begins at the vertical dotted line with the steady-state average indicated by the horizontal gray line. Error bars represent one standard deviation.



	Exit Aceq Yield, Ya_E		Exit Yield, Y_E		Culture Yield, Y_C		Process Yield, Y_P	
	(g aceq /g NAVS fed)		(g acid /g NAVS fed)		(g acid /g NAVS fed)		(g acid /g NAVS fed)	
Train 1	0.140 ± 0.002	vs. T1	0.106 ± 0.001	vs. T1	0.083 ± 0.001	vs. T1	0.072 ± 0.001	vs. T1
Train 2	0.177 ± 0.003	27%	0.138 ± 0.003	31%	0.116 ± 0.003	39%	0.105 ± 0.002	46%
Train 3	0.183 ± 0.003	31%	0.137 ± 0.002	30%	0.114 ± 0.002	38%	0.090 ± 0.001	25%
Train 4	0.129 ± 0.004	–8%	0.109 ± 0.004	3%	0.087 ± 0.004	4%	0.048 ± 0.003	–33%
Train P	0.166 ± 0.002	19%	0.125 ± 0.002	19%	0.103 ± 0.002	24%	0.088 ± 0.001	22%

Figure 6-8. Comparison of yield values for each train. The error bars represent a 95% confidence interval (2 standard deviations). The columns right of the error values represent the improvement relative to Train 1. The feed yield Y_F equals 0.022 g acid/g NAVS fed.

6.3.4. Yield

The exit, culture, and process yields were greatly influenced by the nutrient loading pattern (Figure 6-8). The exit yield Y_E (Equation 6-11) includes acid in the product transfer liquid, waste transfer solids, and liquid samples taken from F2–F4. The exit aceq yield for Trains 1, 2, 3, 4, and P were 0.140, 0.177, 0.183, 0.129, and 0.166 g aceq/g NAVS fed, respectively. Trains 2, 3, and P had exit aceq yields higher than Train 1 by 27%, 31%, and 19%, respectively.

Trains 2 and 3 had statistically identical exit yields (0.138 and 0.137 g acid produced/g NAVS fed, respectively) with Trains 1, 4, and P having yields of 0.106, 0.109, and 0.125 g acid/g NAVS fed, respectively. Trains 2, 3, 4, and P had exit yields higher than the traditional nutrient addition method (Train 1) by 31%, 30%, 3%, and 19%, respectively.

The culture yield Y_C (Equation 6-12) represents the acid *produced* by the microbial cultures, which is equal to the exit yield minus the feed yield. The culture yield Y_C for Trains 1, 2, 3, 4, and P were 0.083, 0.116, 0.114, 0.087, and 0.103 g acid produced/g NAVS fed, respectively. Trains 2, 3, 4, and P had higher culture yields than Train 1 by 39%, 38%, 4%, and 24%, respectively.

This paper introduces *process yield* Y_P (Equation 6-13), which quantifies *only* the acid in the product transfer liquid (L_1) but *not* the acids in the waste transfer solids (S_4). The process yield is of interest because it quantifies the net yield of acid that is sent downstream for concentration and further processing. In a commercial operation, recovering acids from waste transfer solids requires a countercurrent wash. Because the recovered acid is dilute, it will be returned to Fermentor F4. The liquid flows countercurrently relative to the solids, so the recovered acids eventually exit Fermentor F1 and become part of the product transfer liquid (L_1), thus increasing the process yield. In this experiment, no steps were taken to recover acid in the waste transfer solids (S_4) and return it to the fermentation; thus, the reported process yields represent the lower process yield limit. The process yield for Trains 1, 2, 3, 4, and P are 0.072, 0.105, 0.090, 0.048, and 0.088 g acid/g NAVS fed, respectively. Trains 2, 3, and P had higher process

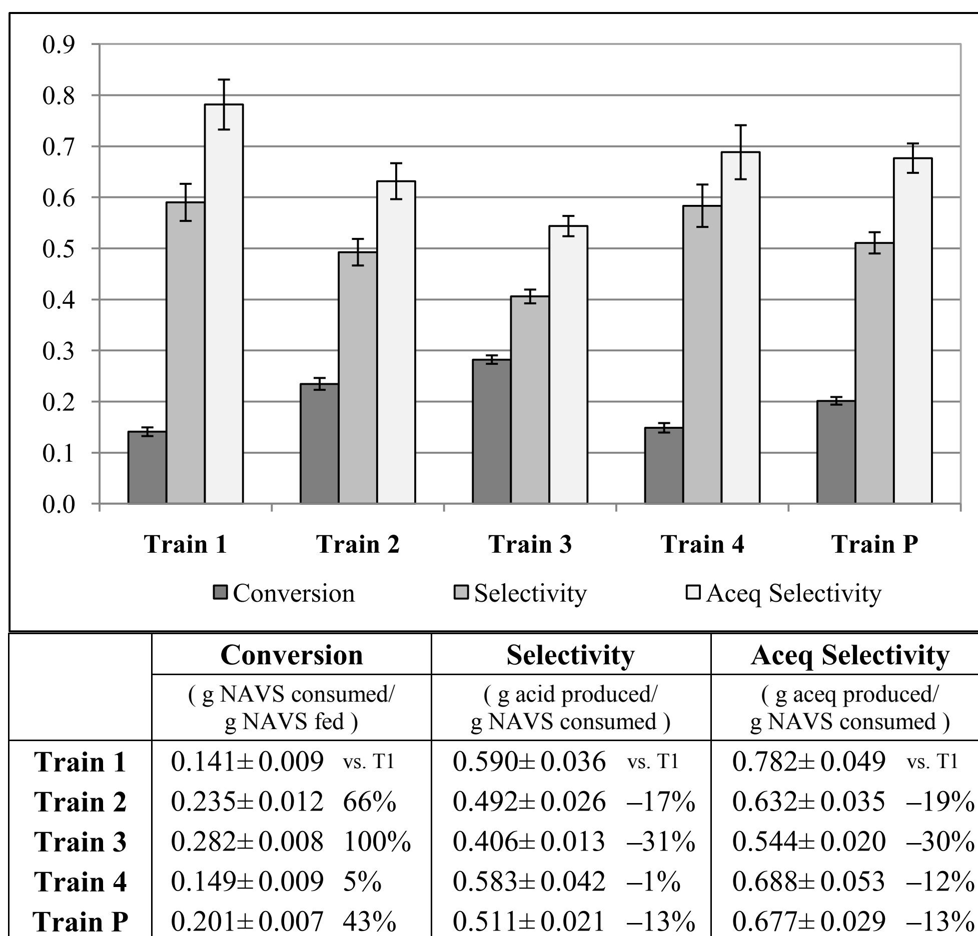
yields than Train 1 by 46%, 25%, and 22%, respectively.

The *exit yield* Y_E represents *all* the acid exiting the fermentation. If the acids in the waste transfer solids (S_4) are counter currently washed with 100% recovery and the acids are returned to Fermentor F4 but impose no additional product inhibition, then all the acids will exit in the product transfer liquid (L_1). In this ideal scenario, the exit yield represents the theoretical upper limit of process yield.

The *process-exit yield ratio* (PE ratio) (Equation 6-27) quantifies the fraction of acid recovered in the product transfer liquid.

$$\text{Process-exit (PE) yield ratio} \equiv \frac{Y_P}{Y_E} \qquad (6\text{-}27)$$

If all acid is recovered from the waste transfer solids, the PE ratio equals 1. The PE ratio for Trains 1, 2, 3, 4, and P were 0.682, 0.761, 0.658, 0.440, and 0.699, respectively. The PE ratios of Trains 2 and 4 were significantly different than Trains 1, 3, and P; thus, PE ratio depends on the nutrient loading pattern. This behavior results from (1) acid in the nutrient feed and (2) changes in solid-liquid separation, which is affected by the extent of digestion. Additionally, the PE ratio (without recovery of acid in waste transfer solids) depends on the solid-liquid separation efficiency, and the relative flow rates of solids and liquids.



	Conversion (g NAVS consumed/ g NAVS fed)		Selectivity (g acid produced/ g NAVS consumed)		Aceq Selectivity (g aceq produced/ g NAVS consumed)	
Train 1	0.141± 0.009	vs. T1	0.590± 0.036	vs. T1	0.782± 0.049	vs. T1
Train 2	0.235± 0.012	66%	0.492± 0.026	–17%	0.632± 0.035	–19%
Train 3	0.282± 0.008	100%	0.406± 0.013	–31%	0.544± 0.020	–30%
Train 4	0.149± 0.009	5%	0.583± 0.042	–1%	0.688± 0.053	–12%
Train P	0.201± 0.007	43%	0.511± 0.021	–13%	0.677± 0.029	–13%

Figure 6-9. Overall conversion and total acid selectivity for steady-state region. The error bars represent a 95% confidence interval (2 standard deviations). The columns to right of the error values represent the improvement relative to Train 1.

6.3.5. Conversion and selectivity

Conversion and selectivity are shown in Figure 6-9. Trains 1, 2, 3, 4, and P had conversions (Equation 6-9) of 0.141, 0.235, 0.282, 0.149, and 0.201 g NAVS consumed/g NAVS fed, respectively. Trains 2, 3, and P had conversions much higher than Train 1 by 66%, 100%, and 43%, respectively. The greatest digestion occurs when both F3 and F4 had near-optimum C/N (25–35 g C_{NA}/g N), which provided nitrogen necessary to digest the most recalcitrant biomass. Train 3 had the highest conversion because it benefits from both near-optimum C/N ratios in F3 and F4, and fresh nutrient feed to F3. Trains 2 had the second highest conversion and benefitted from near-optimum C/N in F2–F4. Train P had higher C/N ratios (~38 g C_{NA}/g N) in the F2–F4, but each fermentor received fresh manure.

Selectivity (Equation 6-14) quantifies the microbial efficiency by reporting the ratio of acid *produced* in fermentation per mass of NAVS consumed; thus, it is equal to the culture yield (Equation 6-12) divided by conversion (Equation 6-9). Trains 1, 2, 3, 4, and P had selectivities of 0.590, 0.492, 0.406, 0.583, 0.511 g acid produced/g NAVS consumed, respectively. Trains 1 and 4 had the highest selectivities, which were statistically similar. Trains 1, 2, 3, 4, and P had aceq selectivities of 0.782, 0.632, 0.544, 0.688, and 0.677 g aceq/g NAVS consumed, respectively. No train had a selectivity or aceq selectivity higher that Train 1. Note, the higher selectivities and aceq selectivities *do not* correspond with the trains that had the highest yields or highest conversion. This observation supports the hypothesis that nutrient-limited environments increase selectivity because stiochiometric ratios are unavailable to create carbon-rich products (e.g., cells, energy-storage compounds, enzymes) that are non-metabolites.

6.4. Conclusions

Nitrogen exists in soluble and insoluble forms traveling in both the transfer solids and transfer liquid streams. Controlling C/N ratios in a countercurrent system is critical to maximizing performance; thus, C/N ratios must be reported to fully understand the context of a fermentation study. To compensate for premature nitrogen loss in the

product transfer liquid from F1 and waste transfer solids from F4, the C/N of the feed should be at, or slightly below, the target C/N (e.g., ~30 g C_{NA}/g N) of the fermentation.

Figures 6-6 to 6-12 show patterns that provide insight about an optimum scenario. Acid in the feed reduces the productivity of the receiving fermentor (Figure 6-5). Performance improves as the C/N ratio of each fermentor approaches the optimum (~30 g C_{NA}/g N) (Figure 6-6). It is better to have a few stages close to the optimum C/N ratio (< 5 C/N points) rather than all stages near the train overall C/N ratio (Trains 2 & 3 vs. Train 1). Non-nitrogen nutrients and/or freshness are critical to optimum performance (Train P vs. Train 1). Nutrients are most critical in the latter stages (Figure 6-6).

Although Trains 2 and 3 had the best yields, no single loading pattern should be used generically as an optimum pattern. The nitrogen properties of the feedstocks, the operating parameters, the solid-liquid separation efficiency, and the nutrient loading pattern influence the behavior of nitrogen in a countercurrent fermentation, which dictates performance. Section 7 introduces a segregated-nitrogen model that may be used to predict/optimize nitrogen concentration profiles for a four-bottle train.

Nutrient feedstocks (e.g., sewage sludge, manure) can contain significant concentrations of organic acids. Characterizing the yield with respect to the feed, exit streams, microbial culture, and product transfer liquid provides greater insight and context to fermentation performance.

7. INVESTIGATION OF NUTRIENT FEEDING STRATEGIES IN A COUNTERCURRENT MIXED-ACID MULTI-STAGED FERMENTATION: DEVELOPMENT OF SEGREGATED-NITROGEN MODEL

The MixAlco^{TM} process is a biorefinery based on the production of carboxylic acids via mixed-culture fermentation. Nitrogen is essential for microbial growth and metabolism, and may exist in soluble (e.g., ammonia) or insoluble forms (e.g., cells). Understanding the dynamics of nitrogen flow in a countercurrent fermentation is necessary to develop control strategies to maximize performance. To estimate nitrogen concentration profiles in a four-stage fermentation train, a mass-balance-based segregated-nitrogen model was developed, which uses separate balances for solid- and liquid-phase nitrogen with nitrogen reaction flux between phases assumed to be zero. Comparison of predictions with measured nitrogen profiles from five trains, each with a different nutrient contacting pattern, shows the segregated-nitrogen model captures basic behavior and is a reasonable tool for estimating nitrogen profiles. The segregated-nitrogen model may be used to (1) estimate optimal nitrogen loading patterns, (2) develop a reaction-based model, (3) understand influence of model inputs (e.g., operating parameters, feedstock properties, nutrient loading pattern) on the steady-state nitrogen profile, and (4) determine the direction of the nitrogen reaction flux between liquid and solid phases.

7.1. Introduction

The MixAlco^{TM} process is a biorefinery that produces carboxylic acids via anaerobic mixed-acid fermentation (Holtzapple and Granda, 2009; Sierra et al., 2008; Smith et al., 2010). The process uses lignocellulose (e.g., high-yield energy crops, wastes) rather than food crops, which are less productive and more expensive (Holtzapple et al., 1999). The carboxylate intermediates are chemically converted into industrial chemicals, solvents, and fuels (e.g., gasoline, alcohols). Granda et al. (2009)

showed that the MixAlco process can produce gasoline for less than $3/gal; thus, the MixAlco™ process is an attractive source of renewable energy.

7.1.1. Need for a nitrogen model

To be economical, the MixAlco process requires high product yields. Granda et al. (2009) used a yield of 0.52 g acid/g non-acid volatile solids (NAVS) fed. To achieve this, optimization of fermentation is essential.

Maintaining sufficient nutrient concentrations and/or proportions is necessary to maximize fermentation performance (Granda et al., 2009). Many studies show that carbon-nitrogen ratio greatly influences fermentation yield. Too much or too little nitrogen can limit fermentation performance (Kayhanian, 1994; Kayhanian and Rich, 1995; Kayhanian and Tchobanglous, 1992; Kim and Holtzapple, 2006; Liu et al., 2008; Marchaim, 1992; Oztekin et al., 2008). Previous mixed-acid fermentations did not quantify or control the carbon-nitrogen ratio (C/N ratio) (Agbogbo and Holtzapple, 2006; Aiello-Mazzarri et al., 2006; Blasig et al., 1992; Coleman, 2007; Domke et al., 2004; Forrest et al., 2010; Fu and Holtzapple, 2009; Fu and Holtzapple, 2010; Fu and Holtzapple, 2010b; Kayhanian and Rich, 1995; Moody, 2006; Ross and Holtzapple, 2001; Thanakoses et al., 2003); thus, these fermentations may have been hindered because of excess or limiting nitrogen.

Countercurrent fermentation allows for both high product concentrations and high conversions. Nitrogen exists in both soluble and insoluble forms; thus, it travels with both the transfer solid and transfer liquid streams. In Section 6, it was shown that nutrient contacting patterns that produced near-optimal carbon-nitrogen (C/N; ~30 g non-acid carbon (C_{NA})/g N) ratios in each stage of a four-staged countercurrent fermentation dramatically improved yield and conversion. Greater improvements in yield are projected if optimal C/N ratios could be maintained in all stages (Section 6).

For similar mixed-acid fermentations making methane or hydrogen, the literature cites a wide range of optimal C/N (10–90 g/g) each with different units (Kim et al., 2006; Liu et al., 2008; Marchaim, 1992). Because 30 is a commonly cited value, for the

purpose of discussion and reference, this paper assumes 30 g C_{NA}/g N is the optimal C/N ratio.

To control an optimal C/N profile, a model is needed to describe the behavior and factors that influence nitrogen flow in a countercurrent fermentation. Additionally, a model will provide a tool to evaluate experiments for nutrient limitations, minimize nutrient costs by maximizing use, and understand the influence of model inputs on nitrogen behavior.

7.1.2. Segregated-nitrogen model

For the purposes of this paper, "nutrient-rich substrate" refers to biomass components that are rich in vitamins, N, P, and minerals (e.g., manure, sewage sludge). "Carbohydrate-rich substrate" refers to biomass components that are rich in carbohydrates – principally cellulose and hemicelluloses – that provide carbon-based energy (e.g., paper, bagasse, corn stover). To obtain an optimum mixture energy and nutrients, mixed-acid fermentations commonly co-digest carbohydrate-rich and nutrient-rich substrates (Agdag and Sponza, 2005; Domke et al., 2004; Rapier, 1995; Sosnowski et al., 2003). The carbohydrate component is the primary substrate for acid production, and is loaded to Fermentor F1; therefore, *only* the nutrient feed point(s) can be controlled. A nitrogen model is needed that describes both the physical flow of nitrogen in the solid and liquid phases *and* the reaction flux of nitrogen between these phases. This paper develops a mass-balance-based segregated-nitrogen model in which the nitrogen in the solid and liquid phases are *segregated* and do not influence each other; thus, the difference between modeled and measured nitrogen concentrations *is* the nitrogen reaction flux between solid and liquid phases.

Model assumptions

This model contains the following assumptions: (1) nitrogen is segregated; soluble nitrogen remains soluble and insoluble nitrogen remains insoluble (i.e., nitrogen reaction flux is zero); (2) nitrogen lost/gained to gaseous phase is negligible; (3) system is at steady state; (4) ideal mixing in each stage; (5) within a stage, the liquid-phase

nitrogen concentration is uniform; thus, the concentration of nitrogen in the free liquid and liquid absorbed in the transfer solids are identical; (6) the solid-phase nitrogen concentration is uniform; and (7) nitrogen reactions within a single phase do not influence the nitrogen flow behavior.

In reality, net nitrogen reaction flux will likely occur between soluble and insoluble nitrogen forms (i.e., Assumption 1 is false). However, Assumption 1 was maintained because sufficient data are not available to theoretically or empirically model the nitrogen reaction flux. In a countercurrent fermentation, nitrogen dynamics is believed to be dominated by non-reactive mass transfer and nitrogen reaction flux between liquid and solid phases. The segregated-nitrogen model will provide insight about (1) the relative contribution of *just* non-reactive mass transfer, and (2) the behavior and direction of the nitrogen reaction flux.

If the pH is below 7.0, which was the case, then nitrogen lost as ammonia gas should be minimal (Assumption 2). Data demonstrated that steady state was achieved and mixing approximated ideal, thus satisfying Assumptions 3 and 4. Theoretically, if ideal mixing is achieved, the nitrogen concentration within a phase should be uniform (Assumptions 5 and 6) nor should intraphase nitrogen reactions influence nitrogen transport (Assumption 7). However, no data are available to validate Assumptions 5–7.

Inputs and outputs

To characterize the feedstocks, the following definitions are employed:

M_{Xi} ≡ the moisture content (g moisture/g wet (as-is) sample) of Stream or Material *Xi*

ν ≡ nitrogen content (g N/ g wet (as-is) sample)

η ≡ soluble nitrogen mass fraction (g soluble N/g total N)

The moisture content and nitrogen content are the mass of moisture and nitrogen, respectively, per mass of material analyzed on a wet (as-is) basis. The soluble nitrogen mass fraction is the mass of soluble nitrogen per total nitrogen. That is, for a given

sample, the fraction of nitrogen that exists in a soluble form relative to the total mass of nitrogen contained in the sample. For example, if the soluble nitrogen fraction is 0.42 g soluble nitrogen/g nitrogen that means that 42% of the nitrogen in the substrate (in this case, wet chicken manure) is of a soluble type (e.g., ammonia) and the remaining 58% is an insoluble type (e.g., bound protein).

Table 7-1. Feedstock properties (Section 6).

	Office Paper	**Fresh Chicken Manure**
Moisture content, M (g H_2O/g wet (as-is) sample)	0.051 ± 0.03	0.660 ± 0.03
Ash content, I (g ash/g dry sample)	0.130 ± 0.06	0.592 ± 0.09
Carbon content, C (g C/g wet (as-is) sample)	36.3 ± 0.8	8.35 ± 0.7
Nitrogen content, N (g N/g wet (as-is) sample)	0.25 ± 0.07	1.10 ± 0.2
Carbon-nitrogen ratio (g C_{NA}/g N)	138.3 ± 43	7.73 ± 0.7
Soluble nitrogen fraction, η (g soluble N/g N)	~0	0.419 ± 0.04

Error values represent one standard deviation

Table 7-1 lists the feedstock properties. Figure 7-1 shows the inputs and outputs for the segregated-nitrogen model. The inputs may be categorized into four groups: (1) feedstock properties (M_{Ni}, ν_{Ni}, η_{Ni}, M_{S0}, ν_{S0}, η_{S0}); (2) nutrient feed strategy (N_i); (3) operating parameters, which dictate the feed rates (S_0 and L_5), the size of the fermentation (F_i), and concentration of solids in each stage ($1–M_{Fi}$); and (4) the solid-liquid separation efficiency, which dictates the moisture contents of the transfer solids (M_{Si}) and liquor (M_{Li}). The solid-liquid separation efficiency depends on the equipment used (centrifuge, screwpress, vacuum filter, etc.) and the degree of digestion of the fermentation solids. The moisture content of each stream defines the proportion of dry solids (i.e., undigested substrate, ash, and cells) and liquid in each stream, thus, providing sufficient data to determine the amount of soluble and insoluble nitrogen in that stream. How the solids and liquids are separated is independent of the model.

Because M_{Si} and M_{Li} are externally influenced, they are considered inputs that must be measured or estimated from other fermentation data.

If the actual stream flowrates are unknown (e.g., fermentation design prior to operation), Method 1 uses an inert-solid mass balance (Figure 7-2; assumes no solid digestion) to estimate the stream flowrates given the feed stream rates (S_0 and L_5) and the moisture content of each stream (M_{Si} and M_{Li}); thus a preliminary output of Method 1 is estimated stream flowrates.

Once the stream flowrates are known (estimated or actual), the system of segregated-nitrogen balances is solved (Figure 7-3). The output (values not circled or boxed in Figure 7-1) is the nitrogen properties (i.e., nitrogen content ν, and soluble nitrogen fraction η) of each transfer solid (S_i) and transfer liquid (L_i) stream. Once the stream nitrogen properties are known, the nitrogen properties of the bulk material in each fermentor may be calculated using Equations 7-6 and 7-7 presented later.

7.1.3. Verification of segregated-nitrogen model

In Section 6, five four-bottle fermentation trains were operated each with a different nutrient-rich substrate contacting pattern (Figure 6-3). Each train was fed a 4:1 ratio (w/w, dry basis) of office paper and fresh (wet) chicken manure. Each train produced a different nitrogen concentration profile, which were used to determine the validity of the segregated-nitrogen model.

Table **7-2** summarizes the input parameters used for Trains 1, 2, 3, 4, and P.

7.2. Materials and methods

In this paper, two prediction methods are used. Figure 7-1 shows the inputs and outputs for both Methods 1 and 2. Method 1 assumes the steady-state stream flowrates (S_i and L_i) are unknown, as would occur when designing a fermentation system. It estimates them with an inert-solids material balance (Section 7.2.1a). Once the steady-state stream flowrates are determined, the values are input into the segregated-nitrogen model (Section 7.2.1b) to determine the nitrogen parameters of the system. Method 2 assumes steady-state stream flowrates are known, which would occur when analyzing an

operating fermentation. In this case, measured stream flowrates are input directly into the inert-nitrogen model, so the inert-solid mass balances of Section 7.2.1a are not required.

7.2.1. Determination of steady-state stream flowrates S_i and L_i

The total mass, total solid, and total moisture mass balances are independent of the nitrogen balances because the relative mass of nitrogen in the fermentation is negligible (~0.1%) relative to the total mass. Two of these three balances may be used to determine the stream flowrates (S_i and L_i). Equations 7-1–3 are the steady-state mass balances written in symbolic form around Fermentor F*i*.

Total mass balance:

$$\frac{d(F_i)}{dt} = 0 = S_{i-1} + L_{i+1} + N_i - S_i - L_i \tag{7-1}$$

Total moisture balance:

$$\frac{d(F_i M_{F_i})}{dt} = 0 = S_{i-1} M_{S_{i-1}} + L_{i+1} M_{L_{i+1}} + N_i M_{N_i} - S_i M_{S_i} - L_i M_{L_i} \tag{7-2}$$

Total dry solids balance:

$$\frac{d\left(F_i\left(1-M_{F_i}\right)\right)}{dt} = 0 = S_{i-1}\left(1-M_{S_{i-1}}\right) + L_{i+1}\left(1-M_{L_{i+1}}\right) + N_i\left(1-M_{N_i}\right) - S_i(1-M_{S_i}) - L_i(1-M_{L_i}) \tag{7-3}$$

The system of equations shown in Figure 7-2 uses the total mass and total moisture balances.

7.2.2. Determination of nitrogen contents and soluble nitrogen fractions

Soluble and insoluble nitrogen mass balances may be written for each stage as follows:

Soluble nitrogen mass balance:

$$\frac{d\left(F_i \nu_{F_i} \eta_{F_i}\right)}{dt} = 0 = S_{i\text{-}1}\nu_{S_{i\text{-}1}}\eta_{S_{i\text{-}1}} + L_{i+1}\nu_{L_{i+1}}\eta_{L_{i+1}} + N_i\nu_{N_i}\eta_{N_i} - S_i\nu_{S_i}\eta_{S_i} - L_i\nu_{L_i}\eta_{L_i} \quad (7\text{-}4)$$

Insoluble nitrogen mass balance:

$$\frac{d\left(F_i \nu_{F_i}(1-\eta_{F_i})\right)}{dt} = 0 = S_{i\text{-}1}\nu_{S_{i\text{-}1}}\left(1 - \eta_{S_{i\text{-}1}}\right) + L_{i+1}\nu_{L_{i+1}}\left(1 - \eta_{L_{i+1}}\right) +$$

$$N_i\nu_{N_i}\left(1 - \eta_{N_i}\right) - S_i\nu_{S_i}\left(1 - \eta_{S_i}\right) - L_i\nu_{L_i}\left(1 - \eta_{L_i}\right) \quad (7\text{-}5)$$

Based on Assumptions 4–6, soluble and insoluble nitrogen in the transfer solids stream (S_i), transfer liquid stream (L_i), and the bulk biomass (F_i) are related by Equations 7-6 and 7-7, respectively.

$$\frac{\nu_{S_i}\eta_{S_i}}{M_{S_i}} = \frac{\nu_{L_i}\eta_{L_i}}{M_{L_i}} = \frac{\nu_{F_i}\eta_{F_i}}{M_{F_i}} = \frac{\text{g soluble nitrogen}}{\text{g liquid}} \quad (7\text{-}6)$$

$$\frac{\nu_{S_i}(1-\eta_{S_i})}{(1-M_{S_i})} = \frac{\nu_{L_i}(1-\eta_{L_i})}{(1-M_{L_i})} = \frac{\nu_{F_i}(1-\eta_{F_i})}{(1-M_{F_i})} = \frac{\text{g insoluble nitrogen}}{\text{g dry solid}} \quad (7\text{-}7)$$

The desired unknowns are ν_{Xi} and η_{Xi}. In each term of Equations 7-4 to 7-7, these quantities are part of the compound variables $\nu_{Xi}\eta_{Xi}$ and $\nu_{Xi}(1-\eta_{Xi})$, which are solved in the system of equations shown in Figure 7-3. From these compound variables, ν_{Xi} and η_{Xi} may be calculated. Once the stream nitrogen properties (ν_{Si}, η_{Si}, ν_{Li}, and η_{Li}) have been determined, Equations 7-6 and 7-7 can be used to determine the nitrogen properties of the bulk biomass (ν_{Fi} and η_{Fi}) in each stage.

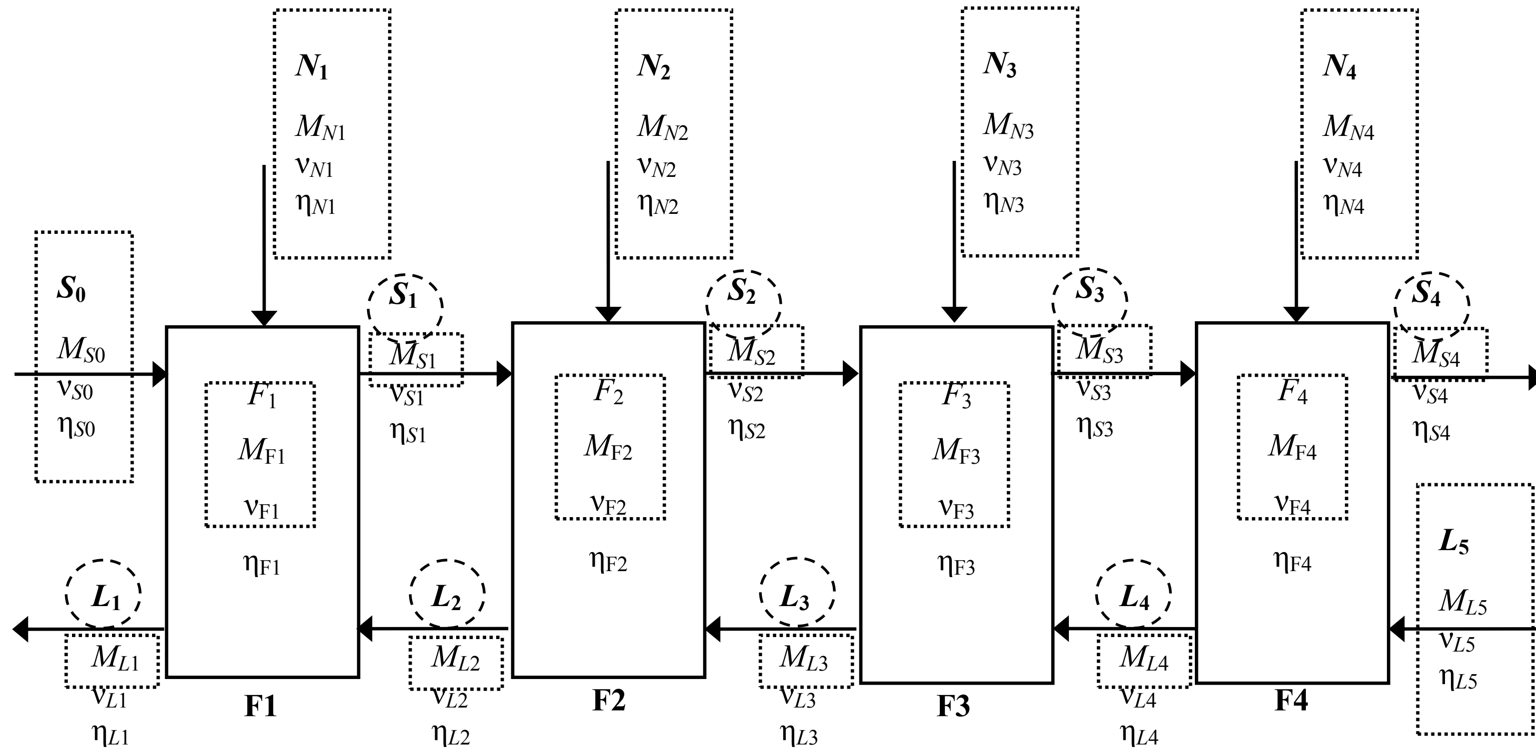


Figure 7-1. Segregated-nitrogen model inputs and outputs for a four-stage countercurrent fermentation. The inputs for Method 1 are indicated by the dotted-line boxes. The inputs for Method 2 include the dotted-line boxes and the stream flowrates in the dashed-line circles. All other values are outputs. S_0, L_5, and N_i are the feed carbohydrate, water, and nutrient stream flowrates (mass/time), respectively. S_i, L_i, and F_i are the transfer solids stream flowrate (mass/time), transfer liquid stream flowrate (mass/time), and total fermentation mass in Fermentor Fi, respectively. M_{Xi}, ν_{Xi}, and η_{Xi} are the moisture content, nitrogen content (w/w) on a wet basis, and the soluble nitrogen fraction of stream/material X_i, respectively.

$$
\underbrace{\begin{bmatrix}
-1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 \\
-M_{L1} & -M_{S1} & M_{L2} & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & -1 & -1 & 1 & 0 & 0 & 0 \\
0 & M_{S1} & -M_{L2} & -M_{S2} & M_{L3} & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & -1 & 1 & 0 \\
0 & 0 & 0 & M_{S2} & -M_{L3} & -M_{S3} & M_{L4} & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 \\
0 & 0 & 0 & 0 & 0 & M_{S3} & -M_{L4} & -M_{S4}
\end{bmatrix}}_{[\,A\,]}
\underbrace{\begin{bmatrix} L_1 \\ S_1 \\ L_2 \\ S_2 \\ L_3 \\ S_3 \\ L_4 \\ S_4 \end{bmatrix}}_{[\,x\,]}
=
\underbrace{\begin{bmatrix} -S_0 - N_1 \\ -S_0 M_{S0} - N_1 M_{N1} \\ -N_2 \\ -N_2 M_{N2} \\ -N_3 \\ -N_3 M_{N3} \\ -L_5 - N_4 \\ -L_5 M_{L5} - N_4 M_{N4} \end{bmatrix}}_{[\,b\,]}
$$

Figure 7-2. System of equations describing the total and moisture mass balances (Equations 7-1 and 7-2) for a four-stage countercurrent fermenation (Method 1). Each dotted-line sub-matrix represents an interface between stages.

$$
[A]\,[x] = [b]
$$

$$
\begin{bmatrix}
-L_1 & 0 & -S_1 & 0 & L_2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & -L_1 & 0 & -S_1 & 0 & L_2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-M_{S1} & 0 & M_{L1} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & M_{S1}-1 & 0 & 1-M_{L1} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & S_1 & 0 & -L_2 & 0 & -S_2 & 0 & L_3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & S_1 & 0 & -L_2 & 0 & -S_2 & 0 & L_3 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & -M_{S2} & 0 & M_{L2} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & M_{S2}-1 & 0 & 1-M_{L2} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & S_2 & 0 & -L_3 & 0 & -S_3 & 0 & L_4 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & S_2 & 0 & -L_3 & 0 & -S_3 & 0 & L_4 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -M_{S3} & 0 & M_{L3} & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & M_{S3}-1 & 0 & 1-M_{L3} & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & S_3 & 0 & -L_4 & 0 & -S_4 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & S_3 & 0 & -L_4 & 0 & -S_4 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -M_{S4} & 0 & M_{L4} & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & M_{S4}-1 & 0 & 1-M_{L4}
\end{bmatrix}
\begin{bmatrix}
\nu_{L1}\eta_{L1} \\
\nu_{L1}(1-\eta_{L1}) \\
\nu_{S1}\eta_{S1} \\
\nu_{S1}(1-\eta_{S1}) \\
\nu_{L2}\eta_{L2} \\
\nu_{L2}(1-\eta_{L2}) \\
\nu_{S2}\eta_{S2} \\
\nu_{S2}(1-\eta_{S2}) \\
\nu_{L3}\eta_{L3} \\
\nu_{L3}(1-\eta_{L3}) \\
\nu_{S3}\eta_{S3} \\
\nu_{S3}(1-\eta_{S3}) \\
\nu_{L4}\eta_{L4} \\
\nu_{L4}(1-\eta_{L4}) \\
\nu_{S4}\eta_{S4} \\
\nu_{S4}(1-\eta_{S4})
\end{bmatrix}
=
\begin{bmatrix}
-S_0\nu_{S0}\eta_{S0} - N_1\nu_1\eta_1 \\
-S_0\nu_{S0}(1-\eta_{S0}) - N_1\nu_1(1-\eta_1) \\
0 \\
0 \\
-N_2\nu_2\eta_2 \\
-N_2\nu_2(1-\eta_2) \\
0 \\
0 \\
-N_3\nu_3\eta_3 \\
-N_3\nu_3(1-\eta_3) \\
0 \\
0 \\
-L_5\nu_{L5}\eta_{L5} - N_4\nu_4\eta_4 \\
-L_5\nu_{L5}(1-\eta_{L5}) - N_4\nu_4(1-\eta_4) \\
0 \\
0
\end{bmatrix}
$$

Figure 7-3. System of equations for nitrogen material balances for a four-staged countercurrent fermentation. Each dotted-line sub-matrix represents an interface between stages.

Table 7-2. Summary of segregated-nitrogen model input parameters (steady state values).

	Stream/ Stage	Train 1 Flowrate (g/T)	Train 1 M_{Xi} (g/g)**	Train 2 Flowrate (g/T)*	Train 2 M_{Xi} (g/g)**	Train 3 Flowrate (g/T)*	Train 3 M_{Xi} (g/g)**	Train 4 Flowrate (g/T)*	Train 4 M_{Xi} (g/g)**	Train P Flowrate (g/T)*	Train P M_{Xi} (g/g)**
Inlet Streams	S_0	35.0	0.070	35.0	0.070	35.0	0.070	35.0	0.070	35.0	0.070
	N_1	24.0	0.660	0.0	0.660	0.0	0.660	0.0	0.660	6.0	0.660
	N_2	0.0	0.660	24.0	0.660	0.0	0.660	0.0	0.660	6.0	0.660
	N_3	0.0	0.660	0.0	0.660	24.0	0.660	0.0	0.660	6.0	0.660
	N_4	0.0	0.660	0.0	0.660	0.0	0.660	24.0	0.660	6.0	0.660
	L_5	300.0	1.000	300	1.000	300.0	1.000	300.0	1.000	300.0	1.000
Transfer Streams	L_1	112.4	0.980	162.6	0.980	159.6	0.980	118.2	0.980	141.2	0.980
	S_1	180.3	0.788	177.9	0.827	166.5	0.829	164.8	0.790	176.3	0.815
	L_2	244.2	0.980	315.3	0.980	298.7	0.980	260.4	0.980	284.1	0.980
	S_2	220.5	0.849	243.6	0.847	208.1	0.854	206.7	0.858	224.4	0.853
	L_3	292.4	0.980	366.9	0.980	348.0	0.980	311.8	0.980	333.5	0.980
	S_3	223.6	0.841	192.8	0.838	208.6	0.841	229.0	0.853	207.2	0.812
	L_4	304.3	0.980	326.5	0.980	337.3	0.980	343.0	0.980	323.7	0.980
	S_4	209.1	0.835	159.4	0.825	162.5	0.842	193.7	0.862	180.3	0.851
Stages	F_1		0.849		0.891		0.891		0.891		0.880
	F_2		0.920		0.929		0.930		0.930		0.928
	F_3		0.929		0.942		0.938		0.938		0.925
	F_4		0.922		0.935		0.945		0.945		0.945

* T = time interval between transfer (~56 h)

**wet basis

7.2.3. Carbon, nitrogen, and moisture contents

In this section, *carbon-nitrogen (C/N) ratio* is defined as the mass of total organic carbon minus the carbon contributed by the carboxylic acids (product) (g non-acid carbon; g C_{NA}) per mass of nitrogen (g N). With respect to acidogens, this definition of C/N ratio characterizes the relative proportion of reactant (energy) per nitrogen (non-energy nutrient). For this study, the organic acids represented 8–18% of the total carbon. If the carbon contributed by the acids is not excluded, the C/N ratio will be overstated, which could lead to over-addition of nitrogen supplements (e.g., urea) (added cost) and sub-optimal performance. Total carbon and total nitrogen contents (g/100 g) were determined by the Texas A&M University Soil, Water, and Forage Testing Lab (College Station, TX), which were measured in a single test using an Elementor Variomax CN. The nitrogen compounds were undefined. Only the total nitrogen content and the partition of nitrogen in the solid and liquid phases were of interest.

Moisture contents (M_{Xi}) were measured in a 105 ºC forced-convection oven (>12 h). Before drying, 3 g $Ca(OH)_2$/100 g wet (as-is) sample was added to ensure all volatile acids were converted to salts and retained during drying.

7.2.4. Method for measuring soluble nitrogen fraction η

To determine the soluble nitrogen fraction η, which is a required parameter for the segregated-nitrogen model (Section 2.1.2), five fresh (wet) chicken manure samples were analyzed. To ensure that all the soluble nitrogen was extracted, each sample was washed a specified number of times. Sample 1 was washed once, Sample 2 was washed twice, and so forth. To perform a washing, 30 g of wet manure was placed into a 1-L centrifuge bottle. For each wash, 500 mL of distilled water was added. The capped bottle was shaken for 10 minutes. The mixture was centrifuged at 4000 rpm for 10 minutes. The liquid was decanted and poured into a single container and combined with liquid from successive washes. The masses of the total collected liquid and remaining cake were measured. Samples of each were analyzed for carbon and nitrogen content (%

w/w). To determine the amount of soluble nitrogen held by the solids, the moisture content of the cake was measured. The soluble nitrogen fraction η was calculated by dividing the nitrogen mass in the liquid, including the moisture in the cake, by the nitrogen mass in the original sample.

7.3. Results and discussion

7.3.1. Soluble nitrogen fraction η

The soluble nitrogen fraction was measured using five samples, each sample receiving a different number of wash cycles (Section 2.1.4). The measured results are shown in Figure 7-4. For Sample 1, η was much lower (0.245) than Samples 2–5 (0.385, 0.438, 0.450, 0.400, respectively), indicating not all soluble nitrogen had dissolved in Sample 1. An average value (0.419 ± 0.08) was calculated from Samples 2–5.

7.3.2. Comparison of predicted and measured nitrogen profiles

Figure 7-5 compares the predicted and measured nitrogen profiles for Trains 1, 2, 3, 4, and P. Both Methods 1 and 2 approximated the measured values. In many cases, the predicted nitrogen concentration is within the measured range. Method 2 is more accurate that Method 1; however, both methods give similar results. For Method 1, the average absolute percent error between measured and predicted nitrogen concentrations for Trains 1, 2, 3, 4, and P were 16%, 30%, 37%, 53%, 30%, respectively. For Method 2, the average absolute percent error between measured and predicted nitrogen concentrations for Trains 1, 2, 3, 4, and P were 13%, 26%, 35%, 64%, 24%, respectively. Because increased conversion decreases the dry solid mass, and so the solid stream flowrates, the discrepancy between Methods 1 and 2 will increase as conversion increases; thus, more error may be observed with Method 1 as the volatile solids loading rate (VSLR, g NAVS/(L_{liq}·d)) and liquid retention time (MRT, days) decrease, which increases conversion.

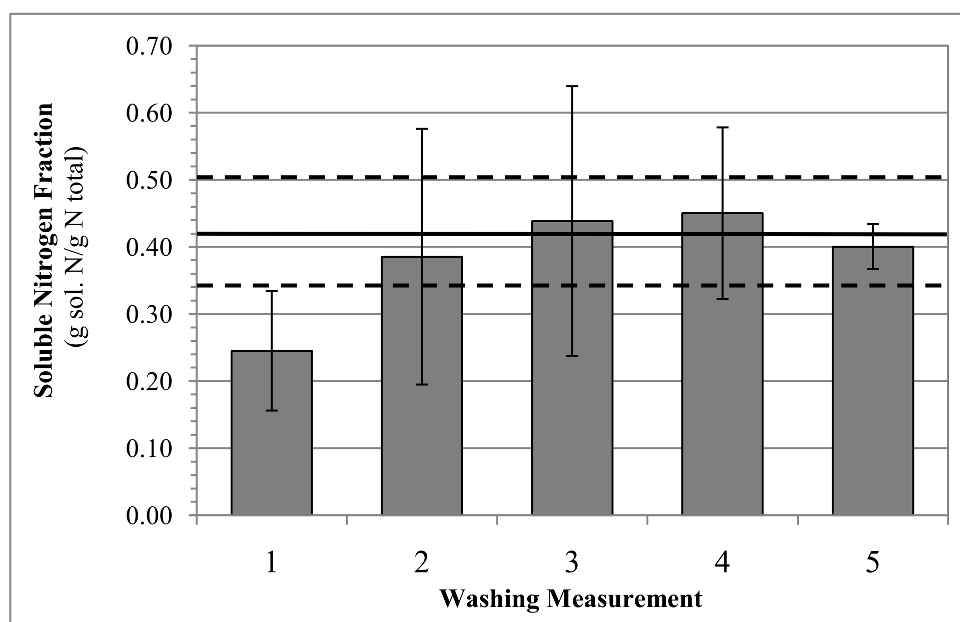


Figure 7-4. Measured soluble nitrogen fraction (Section 2.1.4). The error bars represent the 95% confidence interval. The solid line represents the average (0.419) of Washing Measurements 2–5. The dotted lines represents the 95% confidence interval (±0.08) of the average of Washing Measurements 2–5, which was calculated using sum-of-squared-errors techniques.

The trends of both Methods 1 and 2 match the measured profile trends. For Trains 1, 2, 3, and 4, the fermentor that was fed the nutrient-rich substrate had the highest measured nitrogen concentration. Except for Train 4, both Methods 1 and 2 captured this peak. For all five trains, the measured nitrogen content of the waste transfer solids (S_4) is much greater than the product transfer liquid (L_1). This trend is true for all five trains and is captured by both Methods 1 and 2. Reasonable agreement between predicted and measured shows the segregated-nitrogen model captures basic behavior.

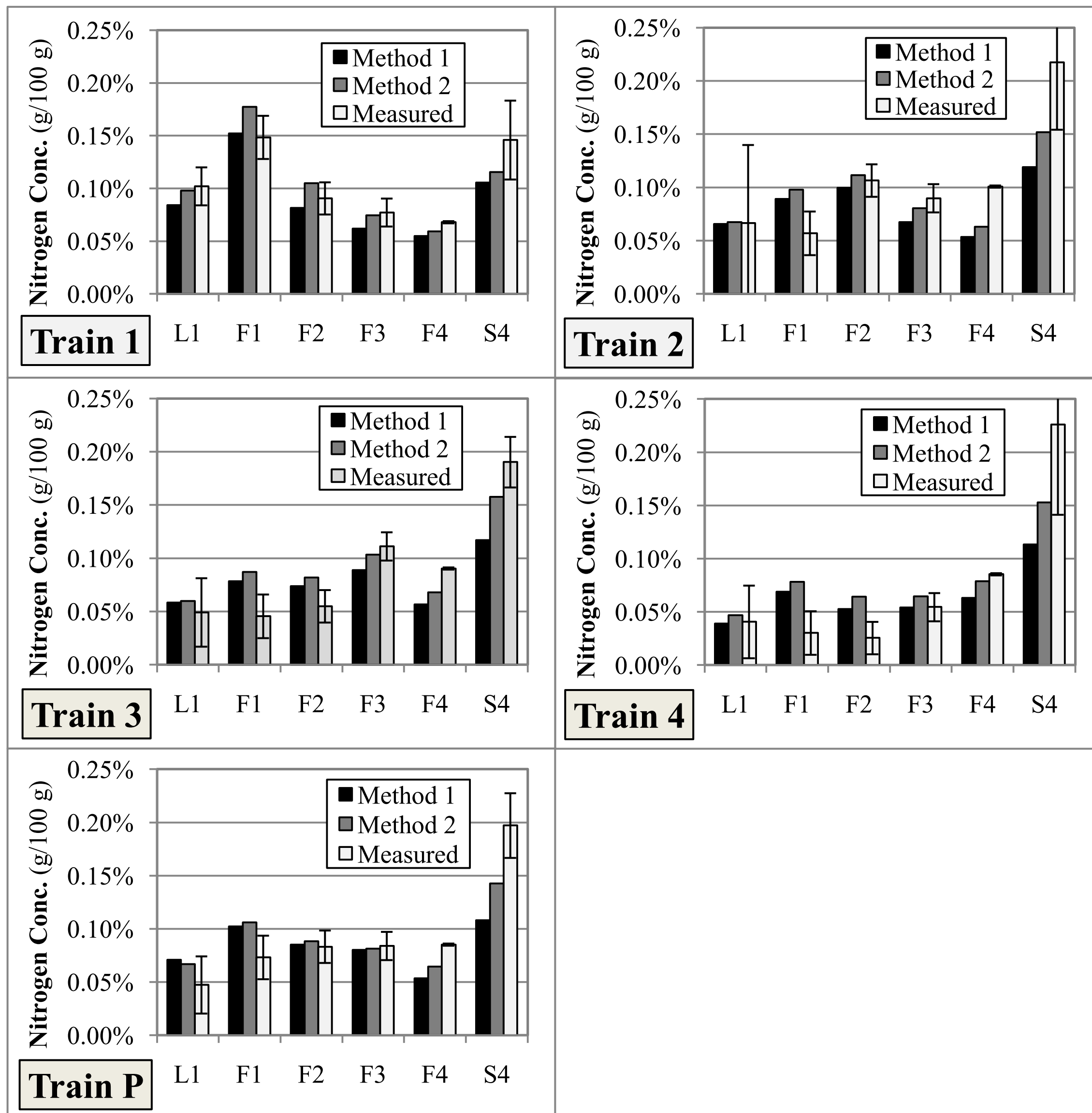


Figure 7-5. Predicted and measured nitrogen concentration profiles for Train 1, 2, 3, 4, and P. Because only two measurements were taken, the error bars represent the range. (Note: Nitrogen concentration is wet basis.)

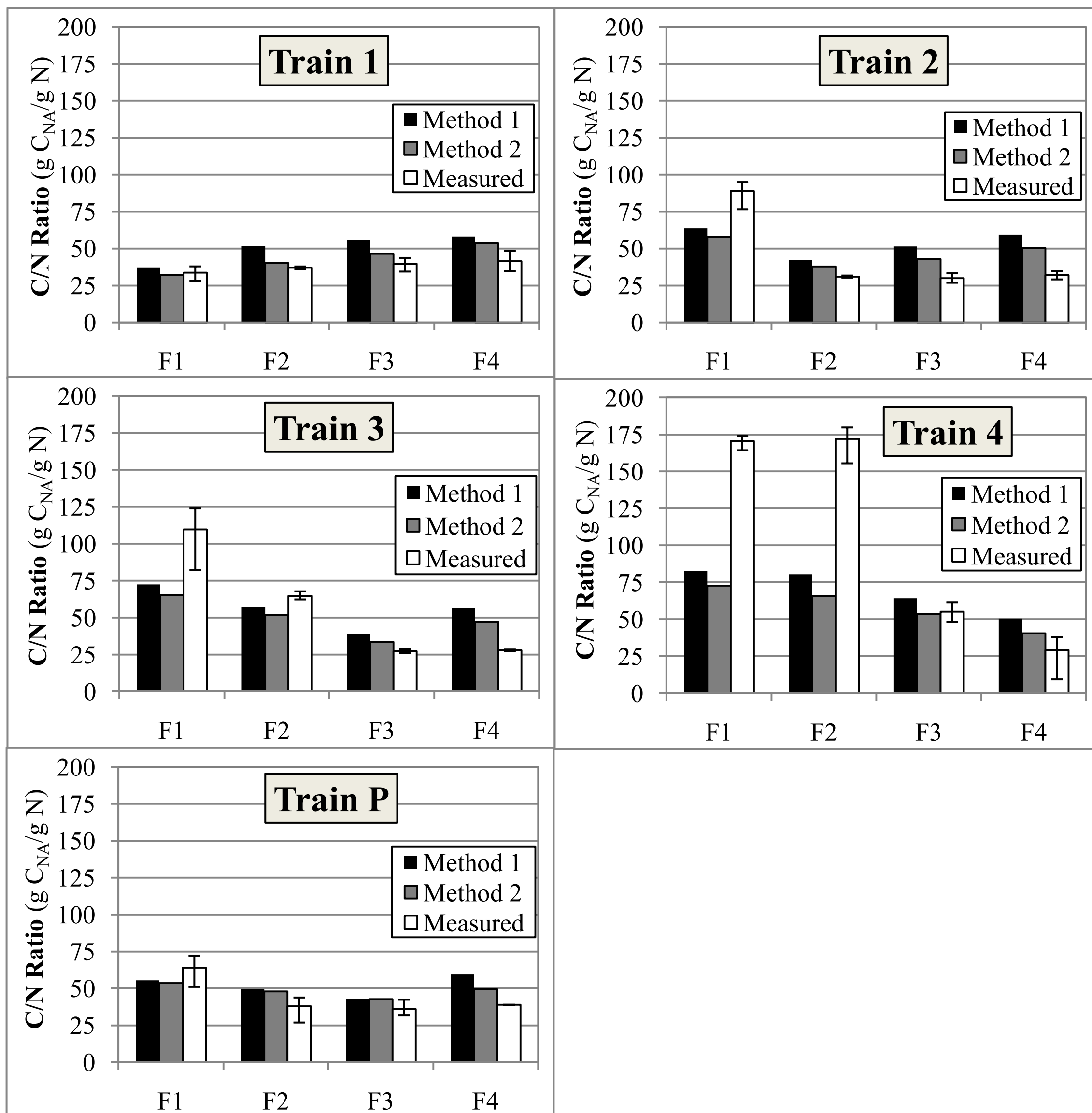


Figure 7-6. Predicted and measured C/N profiles for Trains 1, 2, 3, 4, and P. Because only two measurements were taken, error bars represent the range. (Note: Nitrogen concentration is wet basis.)

Figure 7-6 shows the predicted and measured C/N profiles. Because the non-acid carbon content profile was virtually identical among all five trains (Figure 7-7), the average non-acid carbon content profile of the five trains was used to predict C/N profile. The predicted C/Ns of F1 and F2 of Train 4 had the greatest error; however, Train 4 also had the worst performance of the five trains (Section 6) and was not an optimal nutrient-rich substrate feeding strategy. Except for a few fermentors, the predicted C/N profiles of the better-performing trains (Trains 2, 3, and P) were within 25 C/N points of the measured value; thus, the segregated-nitrogen model is useful for estimating C/N profiles. Because Trains 2, 3, and P approximate the optimal scenario, the discrepancy between the measured and predicted nitrogen profiles (Figure 7-8) of these trains typifies the magnitude of the discrepancy that may be observed in an optimal nutrient-rich substrate feeding strategy, which will be a linear combination of Trains 1, 2, 3, and 4 (See example; Section 3.4).

7.3.3. Model evaluation

Figure 7-8 shows the error (measured minus predicted) profiles for Trains 1, 2, 3, 4, and P, which have a consistent trend among all five trains. In all cases, the measured nitrogen concentration in the product transfer liquid (L_i) and first stages (typically F1 and F2) is less than the predictions. Conversely, the measured concentration in the latter stages (typically F3 and F4) and waste transfer solids (S_4) is greater than the predictions. This diagonal-right error trend can be explained as follows: (1) experimental error, (2) nitrogen lost as gas, and/or (3) reaction between soluble and insoluble forms.

Experimental error – Input stream flowrates and moisture contents were measured with minimal error (typically <2% of value). Further, in a sensitivity analysis in which these values were changed within the error bounds, the diagonal-right trend remained. The nitrogen properties (ν and η) of the feed are less accurate. In a sensitivity analysis in which these values were changed within the error bounds, the trend does not change; therefore, experimental error *does not* account for the diagonal-right error trend.

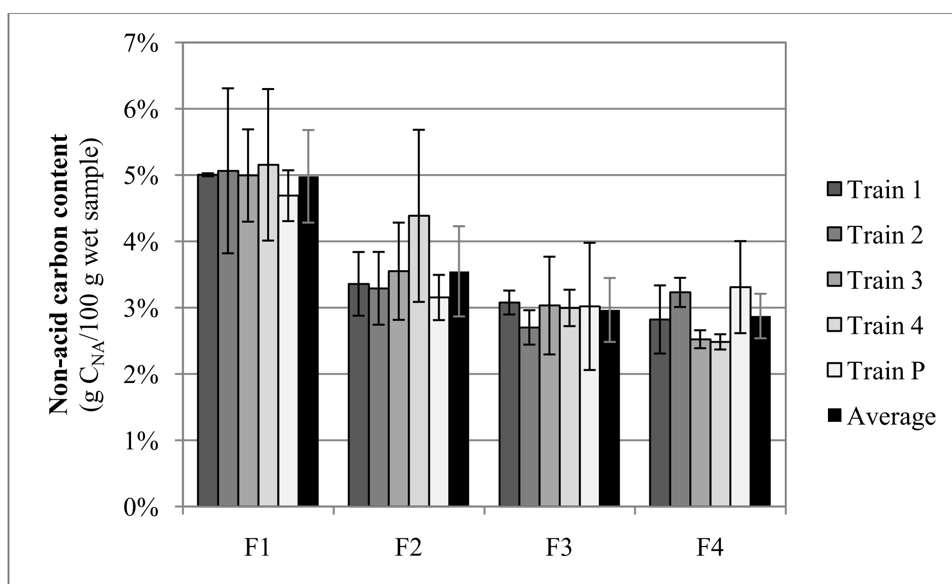


Figure 7-7. Non-acid carbon content profiles for Trains 1, 2, 3, 4, P, and the average profile of those five trains. Because only two measurements were taken, error bars represent the range. (Note: Carbon content is wet basis.)

Nitrogen lost as gas – Because the pH was always below 7, significant loss of nitrogen as ammonia gas is unlikely. Because the fermentation is a reducing environment, nitrogen could not be lost as an oxidized species (e.g., NO_2), so significant loss to gaseous nitrogen is not reasonable. Further, if gaseous nitrogen loss were significant, it would only contribute a negative error profile because the measured nitrogen concentrations would be less than the prediction, which is inconsistent with the diagonal-right error trend.

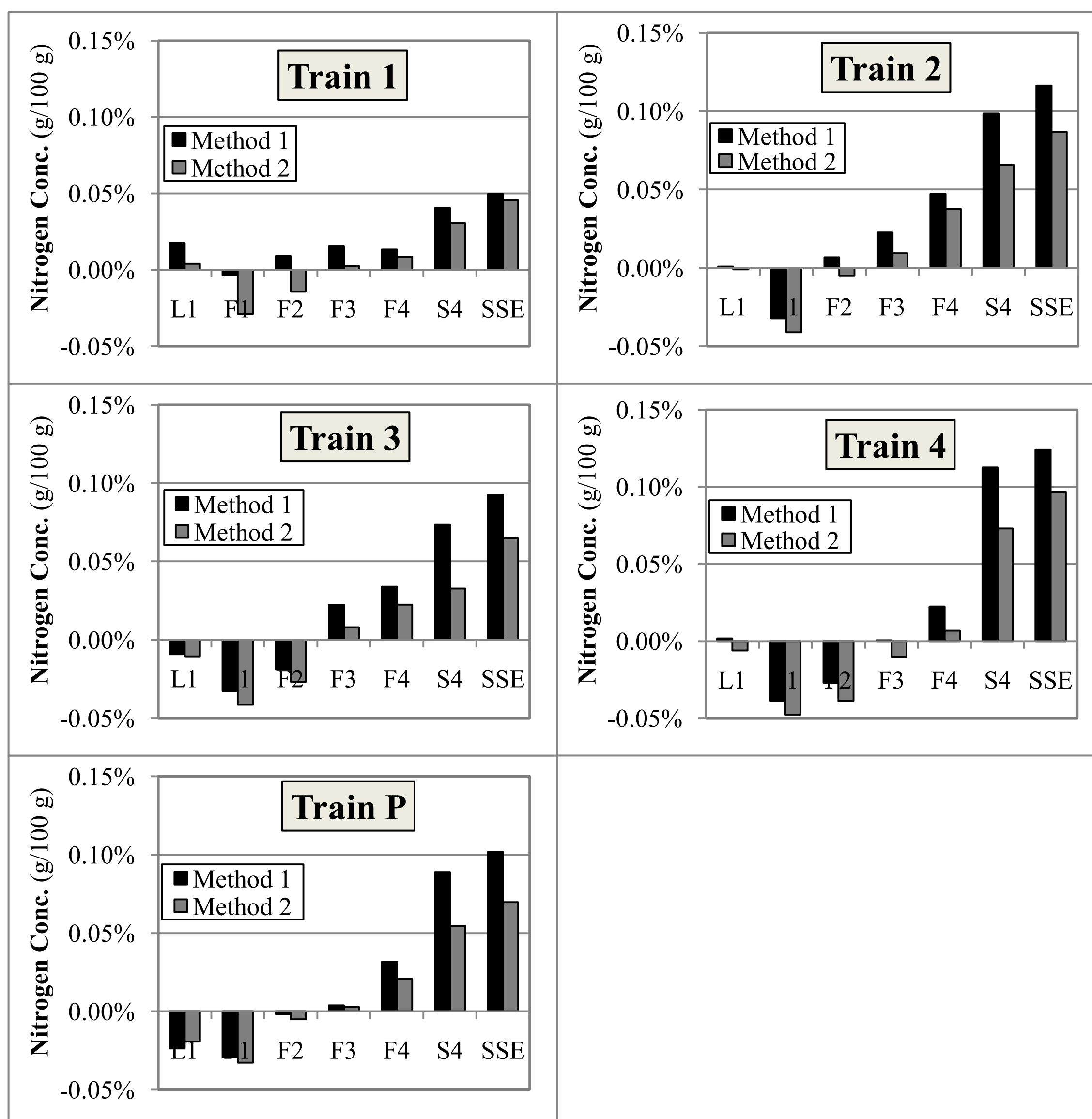


Figure 7-8. Error (measured minus predicted) profiles for Methods 1 and 2. SSE is the sum of squared errors. (Note: Nitrogen concentration is wet basis.)

Reaction between soluble and insoluble forms – The core assumption (Assumption 1) of the model is that soluble and insoluble nitrogen are segregated such that soluble and insoluble nitrogen do not interchange (i.e., nitrogen reaction flux is zero). Violation of this assumption is the most logical explanation. A net reaction flux from soluble to insoluble nitrogen explains the observed diagonal-right error trend, which is consistent with microorganisms metabolizing soluble nitrogen to form cells and insoluble proteins, such as enzymes. The predictions overstate the nitrogen concentration in L_1, F1, and F2 because soluble nitrogen is converted to insoluble nitrogen, which reversed direction leaving these streams and stages with less nitrogen than predicted. Conversely, the predictions understate nitrogen concentrations in F3, F4, and S_4 because the created insoluble nitrogen accumulates in these latter stages. If the net nitrogen reaction flux were from insoluble to soluble, the error profile would flip-flop (diagonal left), which is not observed in Figure 7-8.

The sum of squared errors (SSE) measures the cumulative error between the measured and predicted profiles. As a trend, the SSE increases as the feed point moves from F1 to F4. When the nutrient-rich substrate is feed to F1, a large fraction of the soluble nitrogen is washed out with the product transfer liquid (L_1); thus, there is less soluble nitrogen to be converted to insoluble forms, thereby reducing SSE. By contrast, when the nutrient-rich substrate is fed to F4, the soluble nitrogen travels with the product transfer liquid and has the most time to convert to insoluble forms and reverse its migration, which increases SSE.

The exception to this trend is Train 2, which has a much larger SSE than Train 3. Assuming all error is caused by nitrogen reaction flux, SSE is a gauge of the flux magnitude. Train 2 had a near-optimal measured C/N in F2–F4, C/Ns equal ~30 g C_{NA}/g N. Because of its near-optimal C/N profile, Train 2 produced the highest acid yields of the five trains. These observations reinforce the hypothesis that providing optimal nutrients increases the production of cells and hydrolysis enzymes, which increases the production of metabolites (carboxylic acids).

Method 2, which uses measured stream flows, more accurately predicts measured

nitrogen concentration profiles. If stream flowrates are unknown, Method 1, which estimates the stream flows, may be used to estimate nitrogen profiles. To improve the segregated-nitrogen model, future research should focus on characterizing and modeling the soluble-insoluble reaction flux.

The application of this model is not limited to four-stage countercurrent systems and can be adapted to model *n*-staged systems, as well as to systems with recycle loops. Further, analogous mass-balanced based models could be developed for other critical elements and nutrients (e.g., P and Fe).

7.3.4. Example

The following example explains how the model may be used to determine the optimal nutrient-rich substrate feeding. In a spreadsheet, the system of equations for nitrogen material balances (Figure 7-3) was constructed using the segregated-nitrogen model input parameters for Train 2 (
Table 7-2). The system of equations can be solved using “MMULT” and “MINVERSE” functions in Microsoft Excel. The carbon content profile was assumed to be equal to the average carbon content profile of Trains 1, 2, 3, 4, and P; the carbon content of Fermentors 1–4 was 0.057, 0.042, 0.035, and 0.032 g C_{NA}/g wet biomass, respectively. In the spreadsheet, the C/N ratio profile was calculated from the assumed carbon content profile, and the model-determined nitrogen content profile. To determine the nutrient-rich substrate feeding strategy (i.e., optimal N_1, N_2, N_3, and N_4) that would achieve an optimal C/N profile of 30 g C_{NA}/g N, the sum of squared errors was calculated between the calculated profile and the optimal profile. Then, using the “Solver” tool in Microsoft Excel the sum of squared errors was set to zero by changing the values of N_1, N_2, N_3, and N_4.

The optimal nutrient-rich substrate loading rates for N_1, N_2, N_3, and N_4 was 21.3, 12.7, 2.5, and 8.8 g wet chicken manure/transfer. From this, example it is shown that (1) the optimal nutrient-rich substrate loading pattern *is* a linear combination of Trains 1, 2, 3, and 4 (not all nutrient-rich substrate fed to a single fermentor), and (2) the C/N ratio of

the feed (35.0 g paper, and 45.3 g wet chicken manure; Table 7-1) is 28.1 g C_{NA}/g N, which is less than the target C/N ratio, indicating additional nitrogen must be fed to compensate for premature nitrogen loss in the product liquid and waste solid transfer streams. The U-shaped pattern (i.e., greater nutrient-rich substrate feed in F1 and F4 than F2 and F3, respectively) is unexpected and counterintuitive to the results (Section 6); thus, highlighting the necessity of nutrient transport models for fermentation optimization.

7.4. Conclusions

Nitrogen is a critical element that greatly influences fermentation performance. The segregated-nitrogen model reasonably approximates the measured nitrogen concentration profiles and captures the basic behavior of nitrogen flow in a countercurrent staged fermentation. Therefore, the segregated-nitrogen model may be used to estimate nutrient feeding strategies to achieve an optimal C/N profile, and mathematically understand the influence of input parameters on nitrogen flow. The discrepancies between the model and the data quantify the soluble-insoluble nitrogen reaction flux, and can be used to create a reaction-based model. The data in this section clearly show a net reaction flux from soluble to insoluble nitrogen; however, this may not be true in general. To improve the segregated-nitrogen model, future research should focus on characterizing and modeling the soluble-insoluble reaction flux.

Method 2, which uses measured stream flows, more accurately predicts measured nitrogen concentration profiles. If stream flowrates are unknown, Method 1, which estimates the stream flows, may be used to estimate nitrogen profiles.

The application of this model is not limited to four-stage countercurrent systems and can be adapted to model *n*-staged systems, as well as to systems with recycle loops. Further, analogous mass-balanced based models could be developed for other critical elements and nutrients (e.g., P and Fe).

8. INVESTIGATION OF THE OPTIMAL CARBON-NITROGEN RATIO AND CARBOHYDRATE-NUTRIENT BLEND FOR MIXED-ACID BATCH FERMENTATIONS

To determine the optimal scenario for mixed-acid fermentations, an array of batch fermentations was performed that independently varied the C/N ratio (adjusted using urea) and the blend of carbohydrate (office paper) and nutrient (wet chicken manure). The substrate mass was measured as grams of non-acid volatile solids (NAVS). A blend of 93% paper and 7% wet chicken manure (dry basis) with a C/N ratio of 37 g C_{NA}/g N had the highest culture yield (0.21 g $acid_{produced}$/g $NAVS_{initial}$), total acid productivity (0.84 g $acid_{produced}$/(L_{liq}·d)), and conversion (0.43 g $NAVS_{consumed}$/g $NAVS_{initial}$). Compared to a commonly used blend of 80% paper and 20% wet chicken manure (dry basis, no urea) the culture yield, productivity, and conversion improved 53%, 44%, and 70%, respectively. Selectivity was a strong function of C/N ratio and varied from 0.167 to 0.667 $acid_{produced}$/g $NAVS_{consumed}$. Nitrogen supplementation with urea allows blends with higher percentages of carbohydrate, which improves performance and reduces costs of (1) capital, (2) nutrients, and (3) downstream processing by reducing impurities from nutrient-rich wastes (e.g., manures, sewage sludge).

8.1. Introduction

8.1.1. The MixAlco^{TM} process

The MixAlco^{TM} process is a biorefinery that converts lignocellulose into useful chemicals and fuels. If needed, lime pretreatment is used to increase biomass digestibility. Then, using a mixed culture, the lignocellulose is fermented into two- to seven-carbon carboxylic acids (i.e., volatile fatty acids (VFAs)), which are buffered to create carboxylate salts. The microbial consortia are similar to those found in methane digesters and animal rumen, except methane is inhibited with iodoform (Fu, 2007; Ross,

1998). Compared to ethanol fermentations, the mixed-acid fermentation has several key advantages: (1) robust non-aseptic fermentation, (2) low capital and operating costs, (3) no externally added enzymes, (4) no genetically modified microorganisms or mono-cultures, (5) no contaminates, and (6) thermodynamically favored products (Datta, 1981; Holtzapple and Granda, 2009; Playne, 1980). The fermentation liquor is clarified and concentrated into dried carboxylate salts, a "biocrude" that can be processed into hundreds of chemicals and synthetic hydrocarbon fuels (Smith et al., 2010).

Lignocellulose fermentations are slow (20–60 days) and dilute (20–30 g acid/L_{liq}) thereby requiring very large commercial fermentors (1–10 gal/annual gallon gasoline); thus, improving fermentation performance will significantly reduce costs. To determine the optimal feedstock composition, this paper systematically varied the percentage of carbohydrate-rich and nutrient-rich components, and the carbon-nitrogen (C/N) ratio.

8.1.2. C/N ratio

Nutrients (e.g., vitamins, minerals) are essential in all fermentations. Nitrogen-containing nutrients (e.g., ammonia, vitamins, proteins) have the greatest impact on fermentation performance because nitrogen is required for cell replication, maintenance, metabolism, and production of hydrolysis enzymes (e.g., cellulase). For heterotrophic systems (e.g., composting, dark fermentations), C/N ratios quantify the relative proportion of energy (carbon) to nutrients (nitrogen). For methane and hydrogen fermentations, which also produce carboxylic acids, the literature uses inconsistent units to quantify carbon-nitrogen ratio (e.g., g starch C/g externally added N, g carbohydrate chemical oxygen demand (COD)/total Kjedahl, g COD/g NO_3-N, g total C/g total N (wet and dry basis)) (Kayhanian and Tchobanglous, 1992; Kim et al., 2006; Liu et al., 2008; Marchaim, 1992; Qi et al., 2003). The following discussion explains the appropriate C/N-ratio units for mixed-acid fermentation.

Mixed-acid fermentations are characteristically dirty complex heterogeneous reaction systems that digest carbohydrates, fats, and proteins; thus, quantifying carbon by a *single* reactant (e.g., starch, cellulose) is not accurate or practical (Price, 1985; Qi et

al., 2003). *Chemical oxygen demand* depends of the oxidation state of the carbon compounds (not the amount of carbon); thus, this quantity is ambiguous and does not uniquely describe the carbon content.

Kayhanian (1992) argues that the numerator should quantify the total organic carbon (TOC) minus the carbon contributed by lignin, because lignin is not biodegraded. However, lignin degradation has been observed in rumens, which also contain mixed cultures of acidogens (Barry et al., 1986; Jung et al., 1983; Kondo et al., 1999; Susmel and Stefanon, 1993). Excluding lignin-based carbon *may* understate the effective C/N ratio; however, including it *may* overstate the effective C/N ratio. For our purposes, the C/N ratio should include lignin-based carbon because (1) some lignin can be microbially degraded by mixed cultures, (2) the definition is simpler, and (3) fewer measurements are required. In this study, the point is moot because office paper is the carbohydrate source, which does not contain significant amounts of lignin (<0.5%) (Kayhanian and Tchobanglous, 1992). Additionally, the C/N ratio should quantify the *reactant* per nutrient; thus, the carbon content of the product carboxylic acids should be excluded. In summary, the numerator should quantify the TOC minus the carbon contributed by the carboxylic acids (i.e., VFAs).

With regard to nitrogen content, using *g externally added nitrogen* is not meaningful because the C/N ratio of the actively fermenting bulk material is the measurement of interest. The literature clearly indicates that acidogens can utilize organic nitrogen (e.g., proteins, amino acids) and ammonia (Aiello-Mazzarri et al., 2006; Boone, 1985; Givens and Rulquin, 2004; Hungate, 1966; Leng and Nolan, 1984; Matei and Playne, 1984; Painter, 1970; Wilson et al., 1930; Winter et al., 1964). Nitrate metabolism is common in aerobic and autotrophic bacteria, which are *not present* in mixed-acid fermentations (Eppley et al., 1969; Painter, 1970). Studies about nitrate utilization by anaerobic heterotrophs *suggest* that mixed cultures of acidogens can metabolize and assimilate nitrate. Wilson et al. (1929) concluded that "nitrates cannot be used as the *sole* source of nitrogen by *Clostridium acetobutylicum*." Painter (1970) describes that many bacteria can reduce nitrate for respiration, but fewer can assimilate

nitrate into cell material. Hungate (1966) explains that "nitrate or nitrite can serve as a source of nitrogen for assimilatory reactions of rumen bacteria after being reduced to ammonia." For biomethane fermentations, Qi et al. (2003) report that at C/N ratios above 3.3 g COD/g NO_3-N "denitrification became incomplete"; i.e., nitrate was no longer consumed. Other texts generically discuss anaerobic heterotrophic denitrification, but provide few details to determine applicability to mixed-acid fermentations (Bitton, 2005; Boone, 1985; Stansbury and Whitaker, 1984). Therefore, use of *total nitrogen* may or may not be appropriate; to be definitive, an explicit *in vitro* study should investigate nitrate metabolism and/or assimilation by mixed cultures where methanogens are inhibited.

Use of total Kjeldahl nitrogen (TKN) is reasonable because it quantifies organic nitrogen and ammonia excluding N-O and N-N bonds; thus, only the most microbially beneficial nitrogen is measured (Boone, 1985; Painter, 1970; Wilson et al., 1930). However, with the development of automatic element analyzers, which use Dumas's combustion technique (Etheridge et al., 1998; McGeehan and Naylor, 1988; Sheldrick, 1986), some scientists are moving away from TKN because it is less convenient (i.e., requires more labor) and uses harsh chemicals (Buckee, 1994; Etheridge et al., 1998; Sheldrick, 1986; Sweeney, 1989). Additionally, there are many variations of Kjeldahl's method that use different catalysts and conditions; thus, making it difficult to compare different TKN values (Buckee, 1994). Using animal, plant, and food products, comparative studies of the TKN and Dumas methods show that in most cases, the methods produce nitrogen contents that differ by less than 10% (Buckee, 1994; Etheridge et al., 1998; Simonne et al., 1997); the Dumas value was typically greater. This indicates that most potential feedstocks have few, or no, N-O or N-N bonds (i.e., <10% of total nitrogen). For substances with virtually no inorganic carbon, there is a cost-and-convenience benefit to use total nitrogen measured by the Dumas method (i.e., element analyzer) because both total carbon and total nitrogen are determined from one analysis. For the above-stated reasons, this section uses *total nitrogen* to quantify C/N ratios for mixed-acid fermentations.

For mixed-acid fermentations, C/N ratio is defined as the mass of non-acid organic carbon (g C_{NA}) per total mass of nitrogen (g N), on a wet sample basis. Carbon and nitrogen contents should always be measured on a *wet* or *as-is* basis because (1) the feedstock will be used in this form, (2) drying changes the C and N concentrations, which are needed to predict the fermentation C/N ratio, and (3) the C/N ratio of the feedstock can change because the absolute amount of C and N in the sample can be lost. C/N ratio measurement of feedstocks should be made *post* pretreatment because C and N contents may change because of gaseous and/or leachate losses.

For methane and hydrogen fermentations, the literature cites a wide range of optimal C/N ratios (10–90) with 30 being the most common; however, these values are difficult to compare because the units varied (Kayhanian and Tchobanglous, 1992; Kim et al., 2006; Liu et al., 2008; Marchaim, 1992; Price, 1985; Price and Cheremisinoff, 1985; Yen and Brune, 2007). Smith et al. (2011) showed that productivity increased as C/N ratio approached 30 g C_{NA}/g N; however, no studies have identified an optimal C/N ratio explicitly for carboxylic acid (i.e., VFA) production.

8.1.3. Past nutrient studies

Co-digestion of two or more substrates, especially a carbohydrate-rich substrate with a nutrient-rich substrate, is a common practice in biomethane and mixed-acid fermentations because (1) the blend performs better than each substrate alone, and (2) co-digestion reduces the need for artificial nutrients. Several co-digestion studies have determined optimal proportions for two-substrate systems. Table 8-1 summarizes the optimal blend, based on highest final acid concentration. From Table 8-1, it is clear that different carbohydrate-nutrient pairs have different optimal blends. Each substrate has an inherent carbohydrate and nutrient composition that influences the nutrient profile of the blend. The MixAlco^{TM} process literature has adopted a blend of 80% carbohydrate and 20% nutrient-rich waste (dry mass basis) as a default because supply and transportation of large quantities of nutrient-rich waste were considered impractical (Agbogbo and Holtzapple, 2006; Aiello-Mazzarri et al., 2006; Domke, 1999; Fu, 2007;

Smith et al., 2011; Thanakoses et al., 2003).

Table 8-1. Optimal two-substrate blends based on highest carboxylic acid concentration.

Study	Substrate A	Substrate B	Optimal A/B Blend
Rapier (1995)[A]	MSW	sewage sludge	80/20
Domke (2002)[A]	paper fines	dried biosludge	60/40
Thanakoses (2002) [A]	bagasse	dried chicken manure	40/60
Black (2002)*[A]	corn stover	dried pig manure	40/60
Sanphoti (1997)[M]	MSW	sewage sludge	90/10
Heo et al. (2004)[M]	food waste	WAS	90/10
Yen and Brune (2007)[M]	waste paper	algal sludge	75/25
Callaghan et al. (2002)[M]	cattle slurry	wet chicken manure	75/25
Callaghan et al. (2002)[M]	cattle slurry	fruit and vegetable waste	50/50
Kim et al. (2003)[M]	food waste	sewage sludge	100/0
Kim et al. (2004)[H]	food waste	sewage sludge	100/0

*data published in Thanakoses (2002), MSW = municipal solid waste, WAS = waste activated sludge, A = mixed-acid fermentation, M = biomethane fermentation, H = biohydrogen fermentation

The amount of carbohydrate-rich substrate, nutrient-rich substrate, and artificial nutrients should be engineered to achieve the optimal *proportion* of carbon to macro nutrients (e.g., N, P, K, Ca, Mg, S) (Kayhanian and Rich, 1995; Kayhanian and Tchobanglous, 1992; Price, 1985; Price and Cheremisinoff, 1985). The final micro nutrient (e.g., Cu, Fe, Zn) *concentrations* should be between growth-limiting (i.e., too low) and toxic (i.e., too high) levels (Kayhanian and Rich, 1995; Lin and Lay, 2005). Past mixed-acid fermentations have added artificial nutrients including artificial saliva, NH_4Cl, urea, trace element solutions, Caldwell & Bryant media, and ammonium hydroxide; however, the amounts added were not determined to achieve optimal proportions and concentrations of macro and micro nutrients, respectively (Aiello-Mazzarri et al., 2006; Blasig, 1991; Chan, 2002; Domke, 1999; Fu, 2007; Rapier, 1995).

Urea has been commonly added according to an experimentally determined addition rate rather than to achieve a specified C/N ratio (Agbogbo and Holtzapple, 2006; Aiello-Mazzarri et al., 2006; Chan, 2002; Coleman, 2007; Domke, 1999; Fu, 2007; Ross, 1998; Thanakoses, 2002). Procedure-based urea addition is simple; however, it does not consider the inherent nutrient content of the feedstocks and can lead to false assumptions about nitrogen requirements, and over-addition, especially for batch fermentations. Domke (1999), Thanakoses (2002), and Chan (2002) did similar *batch* fermentations studies to determine the optimal urea addition *rate* ($g/(L_{liq} \cdot d)$). Each investigated addition rates of 0.0, 0.1, and 0.2 g urea/(0.3 L_{liq}) per day or every other day. These studies assumed that *continued* urea addition is necessary, which increases the nitrogen concentration with time, and can increase pH, both of which can adversely affect fermentation performance. Compared to the control, all three studies showed that urea addition increased the final acid concentration; thus, nitrogen supplementation can be beneficial. For Domke (1999) and Thanakoses (2002), the two urea-addition rates achieved statistically similar final acid concentrations indicating nitrogen saturation had occurred in the higher-urea-addition-rate batches. It is possible that nitrogen saturation also occurred in the lower urea addition rate; therefore, for batch fermentation, *continued* urea addition may not have been necessary or optimal.

Rapier (1995) investigated ammonia-nitrogen requirements for a mixed-acid fermentation using 80% municipal solid waste and 20% dry sewage sludge, on a dry basis. Ammonia concentrations of 0, 15, 30, 50, 75, and 100 mM were investigated. His data showed that acid production in the 50-, 75-, and 100-mM ammonia-nitrogen fermentations were initially inhibited; however, after 20 days, these fermentations had acid concentrations higher than the 0-, 15-, and 30-mM ammonia-nitrogen fermentations. The 100-mM ammonia-nitrogen fermentation had the highest acid concentration (19.8 g acid/L_{liq}), which was statistically similar to 50- and 75-mM ammonia-nitrogen fermentations (19.2 and 19.1 g acid/L_{liq}, respectively).

For the 50-, 75-, and 100-mM ammonia-nitrogen fermentations, the initial inhibition can be explained by a basic pH, which likely occurred from the ammonium

hydroxide addition; thus, when using ammonia-nitrogen nutrient supplements (e.g., ammonium hydroxide, urea), the effect of pH must be considered. The similarity in final acid concentration among the 50-, 75-, and 100-mM ammonia-nitrogen fermentations can be explained by one or more of the following: (1) the basic pH promoted ammonia gas formation such that the resulting aqueous ammonia-nitrogen concentrations were virtually identical, (2) the initial C/N ratio for these fermentations were not significantly different, and/or (3) all had excess ammonia. Rapier (1995), Domke (1999), and Thanakoses (2002) did not report initial and final pH, or initial and final C and N content; thus, these explanations are speculative, but illustrate why pH, and C and N contents are critical for data interpretation. This section seeks to determine the optimal C/N ratio, which can then be used to determine the amount of nitrogen supplement required.

8.2. Material and methods

8.2.1. Experiment overview

To determine the optimal scenario, this paper systematically varied the C/N ratio and the blend percentage of paper (P) and wet chicken manure (CM), which is expressed as g P/100 g (P + CM) (dry basis). Figure 8-1 describes the batch fermentations that were measured to characterize the data space. Each of the 30 data points was measured in triplicate. Each fermentation had an equal mass of 15.6 g total carbon and a solid concentration of 100 g VS/L_{liq}, so there were only two independent variables: blend percentage and nitrogen mass. Prior to the experiment, it was hypothesized that the optimum would be between 60 and 90 g P/100 g (P + CM); thus, this range has the highest density of data points.

8.2.2. Fermentation

Table 8-2 lists the feedstock properties, and Table 8-3 shows the amount of each ingredient added to each fermentation.

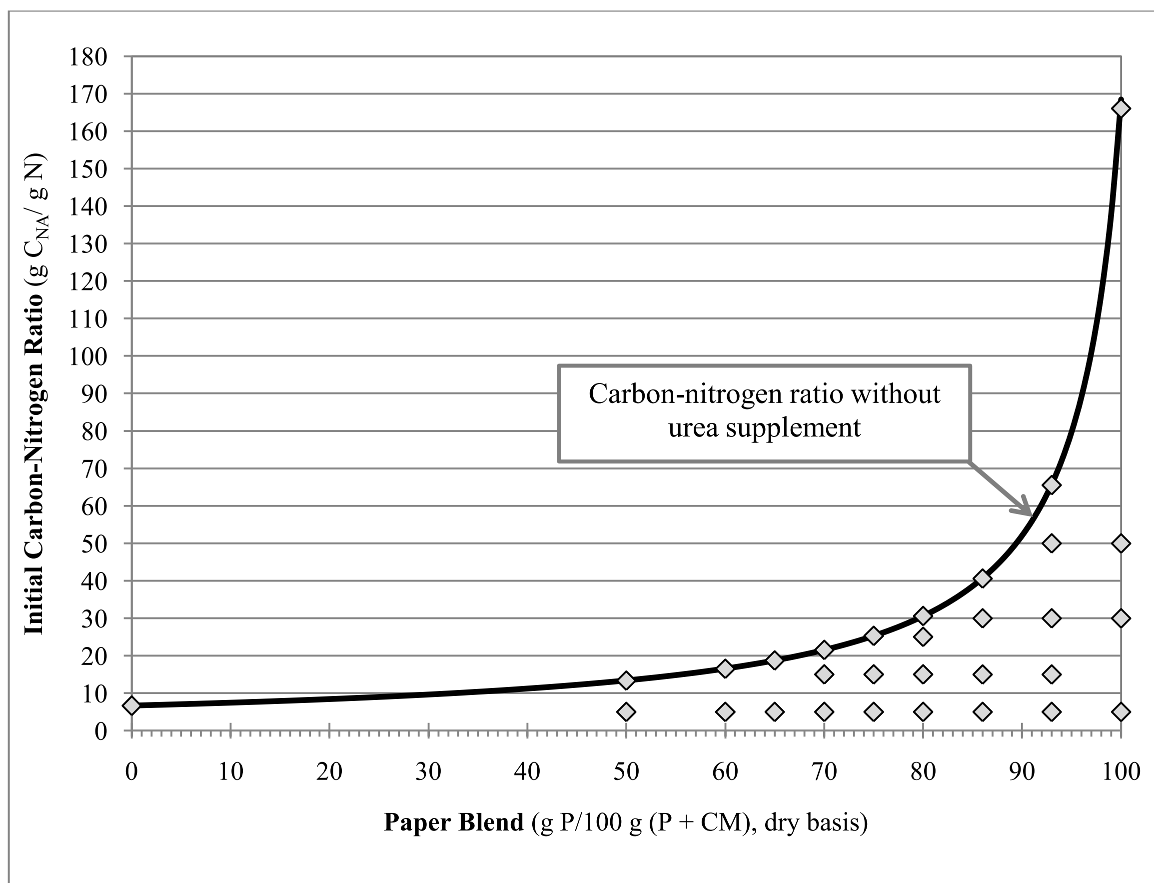


Figure 8-1. Experiment plan. Each diamond represents a batch fermentation used to characterize the data space. P = office paper, CM = wet chicken manure.

Substrates

Shredded office paper (carbohydrate) from Texas A&M University's Recycling Center and wet chicken manure (nutrient) from Feathercrest Farms, L.L.C. (Bryan, TX) were used because they do not require pretreatment. To ensure that homogeneous samples of the initial bulk fermentation mass could be taken, the office paper was milled into a coarse powder (<2 mm diameter particles) using a Thomas-Wiley Laboratory Mill Model 4 with a 2-mm screen (Clarkson and Xiao, 1999). To minimize nutritional

variations among fermentations, the chicken manure was homogenized in a modified 1.5-L food processor, which had a ½-hp motor with a direct drive-shaft. The blend of a paper and wet chicken manure is expressed on a dry basis.

Table 8-2. Feedstock properties.

	Office Paper	Wet Manure	Inocula	Urea
Moisture content, *M* ($g\ H_2O$/100 g wet sample)	4.4 ± 1.0	76.2 ± 3.0	96.0 ± 2.0	0.0
Ash content, *I* (g ash/100 g dry sample)	16.9 ± 0.5	41.7 ± 0.5	40.0 ± 0.5	0.0
Carbon content, C (g C/100 g wet sample)	37.16 ± 0.08	8.48 ± 0.03	1.04 ± 0.01	19.35*
Nitrogen content, N (g N/100 g wet sample)	0.16 ± 0.04	1.31 ± 0.04	0.064 ± 0.01	45.16
Carbon-nitrogen ratio (g C_{NA}/g N)	249.3 ± 55.0	6.5 ± 0.2	16.1 ± 1.8	0.43*

Error values represent one standard deviation
* The carbon in urea converts into CO_2 via hydrolysis; thus, it does not participate in the fermentation and was excluded from C/N-ratio calculations.

Nutrient supplements

To adjust the initial C/N ratio, urea (certified ACS grade, Fisher Chemical cat. No. U15-500) was added. The chicken manure is rich in nitrogen and non-nitrogen nutrients (e.g., vitamins, minerals). No other nutrient supplements (e.g., Caldwell & Bryant media) were added because it was desired to observe the paper blend (g P/100 g (P + CM)) where non-nitrogen nutrients provided by the wet chicken manure limited performance; adding supplemental minerals would interfere with this observation.

Inocula

Each batch fermentation was inoculated with 50 g of strained (1-mm; #18 mesh)

fermentation liquid from a prior office-paper-and-wet-chicken-manure fermentation. The original culture was marine microorganisms from Galveston, TX, which was dominated by *Clostridia* species.

Fermentation media

Deoxygenated water was prepared by boiling deionized water to liberate dissolved oxygen gas. After cooling to room temperature (covered), 0.275 g sodium sulfide and 0.275 g cysteine (i.e., reducing agents) were added per liter of water. The amount of water added to each fermentor was carefully calculated based on the moisture content of other ingredients to achieve 100 g VS/L_{liq}.

Methanogen inhibitor

Methane production is effectively inhibited by iodoform; therefore, each fermentor initially received 120 μL of methane inhibitor (20 g iodoform/L 200-proof ethanol) and then 80 μL daily (Chan, 2002; Ross, 1998).

Buffer and pH

Each batch fermentor was initially loaded with 6.0 g of calcium carbonate. pH was monitored with an Orion model 230A pH Meter.

Table 8-3. Ingredients with initial amounts (as-is basis) added to batch fermentation.

Label	Paper	Urea	Wet Manure	Inocula	Water	$CaCO_3$	Inhibitor
	g	g	g	g	g	g	μL
100-C	39.83	0.00	0.00	50.0	264.8	6.0	120.0
100-50	39.83	0.47	0.00	50.0	264.8	6.0	120.0
100-30	39.83	0.92	0.00	50.0	264.8	6.0	120.0
100-5	39.83	6.51	0.00	50.0	264.8	6.0	120.0
93-C	37.29	0.00	11.30	50.0	251.8	6.0	120.0
93-50	37.29	0.16	11.30	50.0	251.8	6.0	120.0
93-30	37.29	0.61	11.30	50.0	251.8	6.0	120.0
93-15	37.29	1.73	11.30	50.0	251.8	6.0	120.0
93-5	37.29	6.20	11.30	50.0	251.8	6.0	120.0
86-C	34.71	0.00	22.74	50.0	238.5	6.0	120.0
86-30	34.71	0.29	22.74	50.0	238.5	6.0	120.0
86-15	34.71	1.41	22.74	50.0	238.5	6.0	120.0
86-5	34.71	5.89	22.74	50.0	238.5	6.0	120.0
80-C	32.47	0.00	32.68	50.0	227.0	6.0	120.0
80-25	32.47	0.24	32.68	50.0	227.0	6.0	120.0
80-15	32.47	1.14	32.68	50.0	227.0	6.0	120.0
80-5	32.47	5.61	32.68	50.0	27.0	6.0	120.0
75-C	30.59	0.00	41.04	50.0	217.3	6.0	120.0
75-15	30.59	0.91	41.04	50.0	217.3	6.0	120.0
75-5	30.59	5.38	41.04	50.0	217.3	6.0	120.0
70-C	28.69	0.00	49.49	50.0	207.5	6.0	120.0
70-15	28.69	0.68	49.49	50.0	207.5	6.0	120.0
70-5	28.69	5.15	49.49	50.0	207.5	6.0	120.0
65-C	26.77	0.00	58.02	50.0	197.7	6.0	120.0
65-5	26.77	4.92	58.02	50.0	197.7	6.0	120.0
60-C	24.83	0.00	66.63	50.0	187.7	6.0	120.0
60-5	24.83	4.68	66.63	50.0	187.7	6.0	120.0
50-C	20.90	0.00	84.11	50.0	167.4	6.0	120.0
50-5	20.90	4.20	84.11	50.0	167.4	6.0	120.0
0-C	0.00	0.00	176.95	50.0	60.0	6.0	120.0

8.2.3. Fermentation conditions

The fermentors have been previously described by Domke (1999), Ross (1998), Thanakoses (2002), and Aiello-Mazzarri (2006). The fermentors were incubated on a Wheaton Modular Cell Production Apparatus at 1.5 rpm and 40 ºC. To keep the fermentors anaerobic when opened, the gas space was continuously purged with N_2 or CO_2. All batch fermentations were designed to have a VS concentration of 100 g VS/L_{Liq} and a total carbon mass of 15.64 g C. The batch fermentations were run for 32 days with samples taken on Days 0, 7, 14, 22, 28, 30, and 32.

8.2.4. Terms and definitions

The following terms and definitions for batch fermentations are used in this section:

Liquid volume

The liquid volume (LV_{Fi}) of Fermentor *i* is expressed as

$$LV_{Fi} = \frac{F_i M_{Fi}}{\rho_{liq}}\left(\frac{1\text{ L}}{1000\text{ mL}}\right) \tag{8-1}$$

where,

F_i = total fermentation mass (g wet material) in Fermentor *i* (Note: When determining *final* amounts, $F_{i,\text{ final}}$ is the adjusted mass as explained in Section 2.5.)

M_{F_i} = bulk moisture content of material in Fermentor *i* (g liquid/g wet material)

ρ_{liq} = density of liquid (1 g/mL)

Acetic acid equivalents

Acetic acid equivalents were calculated as previously described in Section 6

Carboxylic acids

The mass of carboxylic acids is calculated as follows:

$$A_{F_i} \equiv \text{amount of acid in Fermentor } i \text{ (g)} = \frac{F_i\, M_{F_i} [A]_{F_i}}{\rho_{\text{liq}}} \left(\frac{1 \text{ L}}{1000 \text{ mL}}\right) \quad (8\text{-}2)$$

$$A_{\text{produced}} \text{ (g)} \equiv A_{Fi,\text{ final}} - A_{Fi,\text{ initial}} \quad (8\text{-}3)$$

where, $[A]_{Fi}$ = total acid concentration (g acid/L_{liq}) in Fermentor i

Non-acid volatile solids (NAVS)

Non-acid volatile solids measurements quantify the reactants that can be converted to carboxylic acids. Because mixed-acid fermentation is so adaptable, a wide variety of organic molecules may be reactants (e.g., cellulose, hemicelluloses, starch, sugar, pectin, some lignin, proteins, fats, and other biodegradable materials). In addition to reactants supplied from the feedstock, fermentation products (e.g., cells, extracellular proteins, secreted polysaccharides) can be converted to carboxylic acids.

Volatile solids (VS) are defined as the mass lost when a dry solid is combusted in an ashing oven at 550˚C. The loss of mass is assumed to be strictly from the combustion of organic material. Carboxylic acids retained in the dry sample as a carboxylate salt appear as a VS; thus, to accurately quantify the reactant, the term *non-acid volatile solids (NAVS)* is defined as all the volatile solids *except* the carboxylic acid products. NAVS is calculated using the following equations:

$\text{NAVS}_{Fi,\text{ initial}}$ (g) ≡ initial amount of non-acid volatile solids (NAVS) in Fermentor i (excludes artificial nutrient supplement(s) and buffer)

$$= \textstyle\sum_j X_j\,(1 - M_{Xj})\,(1 - I_{Xj}) - A_{Fi,\text{ initial}} \quad (8\text{-}4)$$

where,

X_j = mass (g wet) of Feedstock j

M_{Xj} = moisture content of Feedstock j (g liquid/g wet material)

I_{Xj} = ash content of Feedstock j (g ash/g dry material)

The volatile solids (VS) concentration (Equation 8-5) has historically been used to quantify the ratio of reactant to liquid volume.

$$\text{VS concentration} \equiv \text{VS}_{Fi,\ \text{initial}} / \text{LV}_{Fi} \tag{8-5}$$

Carboxylic acids are both products and VS, and must be subtracted from the total VS; thus, the NAVS concentration (Equation 8-6) more accurately defines the ratio of reactant (NAVS) to the liquid volume

$$\text{NAVS concentration} \equiv \text{NAVS}_{Fi,\ \text{initial}} / \text{LV}_{Fi} \tag{8-6}$$

Conversion, yield, selectivity, and productivity

The conversion is defined as follows:

$$\text{conversion} \equiv C \equiv \frac{\text{NAVS}_{Fi,\ \text{consumed}}}{\text{NAVS}_{Fi,\ \text{initial}}} \tag{8-7}$$

where, $\text{NAVS}_{Fi,\ \text{consumed}}$ is defined in Section 8.2.6 by Equation 8-14

Because significant quantities of carboxylic acids can be present in the feedstock (e.g., acetyl groups on hemicelluloses, mixed-acids in manure), quantifying yield is complex. To distinguish between carboxylic acids that are *produced* via fermentation and those that merely passed through from input streams to outlet streams, the following definitions are useful:

$$\text{feed yield} \equiv Y_F \equiv \frac{A_{Fi,\ \text{initial}}}{\text{NAVS}_{Fi,\ \text{initial}}} \tag{8-8}$$

$$\text{exit yield} \equiv Y_E \equiv \frac{A_{Fi,\ \text{final}}}{\text{NAVS}_{Fi,\ \text{initial}}} = Y_F + Y_C \tag{8-9}$$

$$\text{culture yield} \equiv Y_C \equiv Y_E - Y_F \equiv \frac{A_{Fi,\ \text{produced}}}{\text{NAVS}_{Fi,\ \text{initial}}} = C \cdot \sigma \tag{8-10}$$

$$\text{process yield (continuous fermentations)} \equiv Y_P \equiv \frac{A_{\text{product liquid}}}{\text{NAVS}_{\text{feed}}} \quad (8\text{-}11)$$

For batch fermentations, the culture yield (Equation 8-10) must be explicitly measured as the difference between the exit and feed yield. It is inaccurate to calculate Y_C by multiplying the difference between the final and initial acid concentrations by the $LV_{initial}$ or LV_{final} because the LV can change with time due to evaporation, which can artificially increase acid concentration.

The following equations define selectivity and productivity:

$$\text{total acid selectivity} \equiv \sigma \equiv \frac{Y_C}{C} \quad (8\text{-}12)$$

$$\text{total acid productivity} \equiv P \equiv \frac{A_{Fi,\ \text{produced}}}{\text{LV}_{Fi} \times \text{time}} \quad (8\text{-}13)$$

8.2.5. Sampling technique

To avoid increasing the substrate concentration, homogeneous samples (i.e., representative mixture of solids and liquid – not just liquid) were taken with a long-handled 1-tsp measuring spoon. The sample mass was determined by weighing the fermentor and its contents before and after sampling. The sample masses removed were depreciated forward in time based on the decay rate of the total fermentation mass between samples; thus, the final fermentation mass was determined as if no samples had been removed. Without accounting for the sample mass removed, conversion would be overstated, and selectivity and closure would be understated.

8.2.6. Analytical methods

Acid concentration and gas composition

Acid concentration and gas composition analysis were determined using an

Agilent 6890 Series Gas Chromatograph (GC) according to methods previously described (Agbogbo, 2005; Aiello-Mazzarri et al., 2006; Chan and Holtzapple, 2003; Fu, 2007; Thanakoses, 2002).

Carbon and nitrogen contents

TOC is preferred in the C/N ratio, but because 99% of the total carbon fed was organic carbon, the added cost of distinguishing the two was not justified. Total C and N contents (g/100 g as-is sample) were determined by Texas A&M University Soil, Water and Forage Testing Lab (College Station, TX) using a Elementor Variomax CN. Based on the C and N contents of the paper, chicken manure, inocula, and urea, the amount of each was calculated to achieve the desired carbon mass, solid concentration, and C/N ratio (Table 8-4). The initial C and N contents were taken before buffer (i.e., $CaCO_3$) addition.

Graphical interpolation

The three-dimensional data were interpolated and plotted using DPlot Version 2.2.5.5 by HydeSoft Computing, L.L.C. (www.DPlot.com). The data points are displayed as small black squares and the interpolation lines are white.

Determination of NAVS consumed

Assuming ash is inert, its initial and final masses are equal. Based on this assumption, the difference in the initial and final mass of dry material (all ingredients included) is because of a change in VS. The g $NAVS_{consumed}$ may be determined by Equation 8-14.

$$\begin{aligned} NAVS_{consumed} &= NAVS_{inital} - NAVS_{final} \\ &= (\Sigma\text{dry solids}_{initial} - \Sigma\text{ash}_{initial} - \Sigma\text{acid}_{initial}) - (\Sigma\text{dry solids}_{final} - \Sigma\text{ash}_{final} - \Sigma\text{acid}_{final}) \\ &= (\Sigma\text{dry solids}_{initial} - \Sigma\text{acid}_{initial}) - (\Sigma\text{dry solids}_{final} - \Sigma\text{acid}_{final}) \end{aligned} \tag{8-14}$$

Moisture and ash contents

Moisture and ash contents were determined by drying samples using a 105 ºC

forced convection oven (>12 h) and a 550 ºC furnace (>3 h), respectively. Before drying, 3 g $Ca(OH)_2$/100 g sample was added to ensure all carboxylic acids were retained during drying.

8.3. Results and discussion

All data are summarized in Tables 8-4 and 8-5. To avoid confusion, data points will be discussed using the fermentation label (e.g., 93-30, 100-C). The first numerical code represents the paper blend (g P/100 g (P + CM); dry basis). The second code represents the target initial C/N ratio. When the second code is a number, it represents the g C_{NA}/g N. Alternatively, it can be a "C," which indicates those fermentations that did not have urea added (i.e., controls). Percentage improvements are expressed relative to 80-C because 80%-carbohydrate-and-20%-nutrient-rich-waste blends have been used as a default in previous mixed-acid fermentations (Agbogbo and Holtzapple, 2006; Aiello-Mazzarri et al., 2006; Domke et al., 2004; Fu, 2007; Smith et al., 2011; Thanakoses et al., 2003).

8.3.1. Acid concentration

Initial acid concentration

Figure 8-2A shows a contour plot of the initial total acid concentration, which is from acid in the inocula and the wet chicken manure. Figure 8-2A has an upward linear trend towards 0 g P/100 g (P + CM) (i.e., 100% wet chicken manure), which indicates the chicken manure had a high acid concentration; thus, initial acid concentration was a function of the paper blend. Pure manure (0-C) had the highest initial acid concentration (24.9 ± 1.3 g/L_{liq}). In contrast, pure paper (100-g P/100 g (P + CM) blends) had the lowest initial acid concentrations (2.0 ± 0.1 g/L_{liq}). Using the data from Figure 8-2A and

Table 8-3, it was determined that the acid concentration of wet manure was 43.3 ± 1.7 g/L_{liq}, which was unexpected because historically feedstocks have been assumed to have a negligible acid content. This discovery affects the experiment design. In retrospect, the ideal experiment should have used a constant mass of non-acid organic carbon (not total carbon) to quantify the feedstock; therefore, the solid concentrations should have been expressed as100 g NAVS/L_{liq} (not 100 g VS/L_{liq}).

In the actual experiment, the discrepancy between the concentration expressed as NAVS or VS is shown in Table 4. In the high-performance region (80–93 g P/100 g (P + CM)), the discrepancy is <4% (relative to 93-30), which is not significant. The greatest discrepancy relative to 93-30 is 0-C, which is ~22% lower. The discrepancy in NAVS concentration in all other low-performance regions is 5–10% less than (relative to 93-30), which is significant; however, these regions are of less importance from an industrial viewpoint. In summary, from a practical perspective, the discrepancies caused by expressing initial substrate concentration as VS rather than NAVS will not have a significant effect on the ultimate objective, which is to recommend operating conditions (i.e., paper blend, C/N ratio) for industrial fermentations.

Table 8-4. C/N ratio, solid concentration, acid concentration, aceq concentration, and pH data for batch fermentations. Errors represent one standard deviation. For performance data, the maximum value is bold.

Label	Paper	Target C/N	Initial C/N	Final C/N	Initial VS Conc.	Initial NAVS Conc.	Initial Acid Conc.	Final Acid Conc.	Final Aceq. Conc.	Initial pH	Final pH
units	g/100 g	g C_{NA}/g N	g C_{NA}/g N	g C_{NA}/g N	g VS/L_{liq}	g NAVS/L_{liq}	g acid/L_{liq}	g acid/L_{liq}	g aceq/L_{liq}	pH	pH
100-C	100	166.0	107.3 ± 46.0	23.1 ± 8.1	100 ± 1.5	98.1 ± 1.5	1.9 ± 0.1	5.5 ± 1.6	6.4 ± 1.6	7.83 ± 0.2	6.46 ± 0.3
100-50	100	49.9	53.0 ± 1.2	30.2 ± 16.4	100 ± 1.5	97.9 ± 1.5	2.0 ± 0.1	6.0 ± 3.3	7.0 ± 3.6	7.84 ± 0.2	6.69 ± 0.4
100-30	100	30.0	36.3 ± 2.8	11.8 ± 3.2	100 ± 1.5	97.9 ± 1.5	2.1 ± 0.1	5.4 ± 1.5	6.4 ± 1.5	7.93 ± 0.2	6.89 ± 0.3
100-5	100	5.0	6.4 ± 0.4	4.7 ± 3.9	100 ± 1.5	98.0 ± 1.5	2.0 ± 0.0	4.4 ± 0.5	5.0 ± 0.6	7.94 ± 0.2	8.86 ± 0.3
93-C	93	65.5	51.0 ± 5.2	27.8 ± 6.6	100 ± 1.5	96.8 ± 1.5	3.2 ± 0.2	22.0 ± 1.5	26.8 ± 1.9	7.65 ± 0.2	5.77 ± 0.1
93-50	93	50.0	48.7 ± 4.7	12.1 ± 14.0	100 ± 1.5	96.7 ± 1.5	3.3 ± 0.3	26.3 ± 1.8	32.6 ± 2.1	7.70 ± 0.2	5.54 ± 0.3
93-30	93	30.0	36.9 ± 1.6	21.0 ± 5.2	100 ± 1.5	96.8 ± 1.5	3.2 ± 0.1	**30.2** ± 2.0	**38.5** ± 2.2	7.77 ± 0.2	5.48 ± 0.3
93-15	93	15.0	21.7 ± 1.6	14.2 ± 9.3	100 ± 1.5	96.6 ± 1.5	3.4 ± 0.1	23.9 ± 5.0	33.9 ± 6.6	7.78 ± 0.2	5.92 ± 0.1
93-5	93	5.0	7.2 ± 0.3	13.1 ± 7.7	100 ± 1.5	96.7 ± 1.5	3.3 ± 0.1	9.2 ± 1.6	10.5 ± 1.6	7.84 ± 0.2	8.72 ± 0.2
86-C	86	40.6	33.8 ± 2.6	15.9 ± 6.3	100 ± 1.5	95.5 ± 1.5	4.5 ± 0.3	23.1 ± 0.6	28.9 ± 0.5	7.52 ± 0.2	5.66 ± 0.3
86-30	86	30.0	30.7 ± 2.5	19.7 ± 4.1	100 ± 1.5	95.4 ± 1.5	4.6 ± 0.0	24.9 ± 1.1	30.8 ± 1.1	7.65 ± 0.2	5.64 ± 0.2
86-15	86	15.0	19.8 ± 3.0	10.8 ± 0.6	100 ± 1.5	95.4 ± 1.5	4.6 ± 0.3	25.1 ± 1.2	32.0 ± 2.4	7.65 ± 0.2	5.88 ± 0.1
86-5	86	5.0	5.3 ± 2.7	5.6 ± 2.7	100 ± 1.5	96.1 ± 2.1	3.9 ± 1.5	11.1 ± 2.5	12.5 ± 2.8	7.77 ± 0.2	8.64 ± 0.2
80-C	80	30.5	26.7 ± 1.7	16.5 ± 1.8	100 ± 1.5	94.2 ± 1.5	5.8 ± 0.2	23.3 ± 1.1	29.7 ± 1.1	7.53 ± 0.2	5.75 ± 0.3
80-25	80	25.0	23.9 ± 2.5	14.5 ± 1.9	100 ± 1.5	94.3 ± 1.5	5.7 ± 0.3	24.6 ± 0.4	30.8 ± 0.6	7.74 ± 0.2	5.77 ± 0.3
80-15	80	15.0	18.6 ± 4.1	10.2 ± 2.8	100 ± 1.5	94.4 ± 1.5	5.6 ± 0.2	24.1 ± 1.0	30.9 ± 1.9	7.81 ± 0.2	6.14 ± 0.4
80-5	80	5.0	7.7 ± 1.1	6.8 ± 2.3	100 ± 1.5	94.4 ± 1.5	5.6 ± 0.2	13.7 ± 1.2	15.3 ± 1.3	7.66 ± 0.2	8.59 ± 0.1
75-C	75	25.3	20.7 ± 0.5	9.9 ± 6.9	100 ± 1.5	93.2 ± 1.5	6.8 ± 0.3	24.4 ± 0.5	30.6 ± 0.6	7.41 ± 0.2	5.95 ± 0.1
75-15	75	15.0	15.9 ± 2.3	7.7 ± 3.7	100 ± 1.5	93.2 ± 1.6	6.8 ± 0.5	24.2 ± 0.7	30.9 ± 0.8	7.65 ± 0.2	6.09 ± 0.1
75-5	75	5.0	7.1 ± 0.8	6.6 ± 3.6	100 ± 1.5	93.4 ± 1.5	6.6 ± 0.2	14.3 ± 0.5	16.2 ± 0.6	7.69 ± 0.2	8.61 ± 0.1
70-C	70	21.5	19.0 ± 2.1	13.5 ± 0.3	100 ± 1.5	92.4 ± 1.6	7.6 ± 0.4	24.8 ± 0.2	30.6 ± 0.5	7.45 ± 0.2	6.05 ± 0.1
70-15	70	15.0	15.8 ± 0.8	10.2 ± 0.1	100 ± 1.5	92.0 ± 1.7	8.0 ± 0.7	25.3 ± 0.6	31.7 ± 0.4	7.68 ± 0.2	6.12 ± 0.1
70-5	70	5.0	8.0 ± 0.7	6.4 ± 0.8	100 ± 1.5	92.2 ± 1.5	7.8 ± 0.3	16.8 ± 1.2	19.0 ± 1.3	7.79 ± 0.2	8.54 ± 0.1
65-C	65	18.7	15.3 ± 0.6	11.6 ± 1.1	100 ± 1.5	90.9 ± 1.8	9.1 ± 0.9	25.6 ± 1.4	31.5 ± 1.5	7.50 ± 0.2	6.11 ± 0.2
65-5	65	5.0	7.3 ± 0.2	4.4 ± 2.0	100 ± 1.5	91.2 ± 1.5	8.8 ± 0.3	18.1 ± 0.9	20.9 ± 1.1	7.72 ± 0.2	8.58 ± 0.2
60-C	60	16.5	13.6 ± 0.3	11.0 ± 1.3	100 ± 1.5	90.2 ± 1.6	9.8 ± 0.6	26.4 ± 0.8	31.9 ± 1.8	7.45 ± 0.2	6.19 ± 0.2
60-5	60	5.0	7.2 ± 1.4	5.5 ± 0.7	100 ± 1.5	90.0 ± 1.6	10.0 ± 0.6	18.8 ± 2.6	21.3 ± 2.6	7.60 ± 0.2	8.55 ± 0.2
50-C	50	13.4	10.5 ± 0.5	9.1 ± 0.4	100 ± 1.5	87.5 ± 1.6	12.5 ± 0.6	24.9 ± 1.4	29.6 ± 1.7	7.47 ± 0.2	6.45 ± 0.2
50-5	50	5.0	6.7 ± 0.9	5.6 ± 0.4	100 ± 1.5	88.1 ± 1.8	11.9 ± 1.0	20.4 ± 1.1	23.7 ± 1.2	7.64 ± 0.2	8.57 ± 0.1
0-C	0	6.6	5.0 ± 0.2	4.5 ± 0.1	100 ± 1.5	75.1 ± 2.0	**24.9** ± 1.3	**30.3** ± 1.0	34.2 ± 1.0	7.71 ± 0.2	7.85 ± 0.2

Table 8-5. Yield, productivity, conversion, selectivity, and closure data for batch fermentation. Errors represent one standard deviation. For performance data, the maximum value is bold.

Label	Feed Yield Y_F	Exit Yield Y_E	Culture Yield Y_C	Productivity P	Culture Aceq Yield $Y_{C, aceq}$	Conversion C	Selectivity σ	Closure
units	g acid/g $NAVS_{initial}$	g acid/g $NAVS_{initial}$	g $acid_{produced}$/g $NAVS_{initial}$	g $acid_{produced}$/ $(L_{liq.} \cdot d)$	g $aceq_{produced}$/g $NAVS_{initial}$	g $NAVS_{consumed}$/ g $NAVS_{initial}$	g $acid_{produced}$/ g $NAVS_{consumed}$	g out/g in (dry basis)
100-C	0.016 ± 0.00	0.042 ± 0.01	0.026 ± 0.01	0.11 ± 0.05	0.029 ± 0.01	0.043 ± 0.02	0.603 ± 0.39	1.06 ± 0.03
100-50	0.016 ± 0.00	0.046 ± 0.02	0.030 ± 0.02	0.12 ± 0.10	0.033 ± 0.03	0.052 ± 0.03	0.565 ± 0.56	1.05 ± 0.04
100-30	0.017 ± 0.00	0.043 ± 0.02	0.026 ± 0.01	0.10 ± 0.05	0.029 ± 0.02	0.095 ± 0.02	0.275 ± 0.17	1.01 ± 0.03
100-5	0.014 ± 0.00	0.031 ± 0.01	0.017 ± 0.01	0.08 ± 0.02	0.016 ± 0.01	0.099 ± 0.02	0.167 ± 0.07	0.97 ± 0.03
93-C	0.027 ± 0.00	0.176 ± 0.04	0.150 ± 0.03	0.59 ± 0.05	0.182 ± 0.04	0.276 ± 0.07	0.542 ± 0.19	0.99 ± 0.04
93-50	0.028 ± 0.00	0.216 ± 0.03	0.188 ± 0.03	0.72 ± 0.05	0.233 ± 0.03	0.350 ± 0.05	0.536 ± 0.11	0.98 ± 0.02
93-30	0.027 ± 0.00	0.239 ± 0.03	**0.213** ± 0.02	**0.84** ± 0.06	**0.273** ± 0.03	**0.429** ± 0.05	0.496 ± 0.08	0.94 ± 0.01
93-15	0.027 ± 0.00	0.190 ± 0.07	0.163 ± 0.07	0.64 ± 0.16	0.235 ± 0.09	0.390 ± 0.13	0.418 ± 0.22	0.93 ± 0.02
93-5	0.023 ± 0.00	0.064 ± 0.01	0.040 ± 0.01	0.18 ± 0.05	0.044 ± 0.01	0.129 ± 0.02	0.312 ± 0.10	0.96 ± 0.03
86-C	0.038 ± 0.00	0.195 ± 0.03	0.157 ± 0.03	0.58 ± 0.01	0.196 ± 0.04	0.237 ± 0.01	**0.663** ± 0.13	1.02 ± 0.03
86-30	0.039 ± 0.01	0.202 ± 0.03	0.163 ± 0.03	0.63 ± 0.03	0.202 ± 0.03	0.291 ± 0.03	0.561 ± 0.12	0.99 ± 0.02
86-15	0.038 ± 0.01	0.201 ± 0.04	0.164 ± 0.04	0.64 ± 0.04	0.212 ± 0.06	0.341 ± 0.03	0.480 ± 0.12	0.96 ± 0.02
86-5	0.029 ± 0.01	0.078 ± 0.03	0.050 ± 0.01	0.23 ± 0.03	0.052 ± 0.01	0.134 ± 0.02	0.373 ± 0.11	0.96 ± 0.03
80-C	0.049 ± 0.01	0.197 ± 0.04	0.148 ± 0.04	0.55 ± 0.04	0.192 ± 0.04	0.252 ± 0.06	0.587 ± 0.20	1.01 ± 0.04
80-25	0.049 ± 0.01	0.204 ± 0.04	0.155 ± 0.03	0.59 ± 0.01	0.196 ± 0.04	0.296 ± 0.02	0.523 ± 0.10	0.98 ± 0.03
80-15	0.047 ± 0.01	0.186 ± 0.03	0.139 ± 0.02	0.58 ± 0.04	0.184 ± 0.03	0.332 ± 0.00	0.418 ± 0.07	0.95 ± 0.05
80-5	0.042 ± 0.01	0.098 ± 0.01	0.056 ± 0.00	0.25 ± 0.04	0.059 ± 0.00	0.155 ± 0.03	0.361 ± 0.08	0.95 ± 0.03
75-C	0.059 ± 0.01	0.206 ± 0.04	0.147 ± 0.03	0.55 ± 0.02	0.188 ± 0.04	0.244 ± 0.05	0.601 ± 0.17	1.01 ± 0.04
75-15	0.058 ± 0.01	0.196 ± 0.03	0.138 ± 0.02	0.54 ± 0.01	0.182 ± 0.02	0.284 ± 0.08	0.486 ± 0.16	0.98 ± 0.09
75-5	0.050 ± 0.01	0.105 ± 0.01	0.055 ± 0.01	0.24 ± 0.02	0.059 ± 0.00	0.135 ± 0.02	0.407 ± 0.08	0.97 ± 0.01
70-C	0.068 ± 0.01	0.210 ± 0.03	0.142 ± 0.02	0.54 ± 0.01	0.180 ± 0.03	0.255 ± 0.03	0.558 ± 0.09	1.00 ± 0.00
70-15	0.070 ± 0.01	0.200 ± 0.04	0.130 ± 0.03	0.54 ± 0.03	0.172 ± 0.03	0.241 ± 0.07	0.542 ± 0.20	1.03 ± 0.02
70-5	0.060 ± 0.01	0.124 ± 0.01	0.064 ± 0.01	0.28 ± 0.04	0.070 ± 0.01	0.129 ± 0.04	0.499 ± 0.16	0.99 ± 0.04
65-C	0.083 ± 0.02	0.223 ± 0.02	0.140 ± 0.00	0.52 ± 0.07	0.176 ± 0.01	0.248 ± 0.06	0.563 ± 0.13	1.00 ± 0.05
65-5	0.069 ± 0.01	0.138 ± 0.02	0.069 ± 0.01	0.29 ± 0.02	0.076 ± 0.01	0.231 ± 0.09	0.298 ± 0.12	0.91 ± 0.08
60-C	0.090 ± 0.01	0.223 ± 0.03	0.133 ± 0.02	0.52 ± 0.01	0.168 ± 0.02	0.266 ± 0.01	0.500 ± 0.07	0.97 ± 0.01
60-5	0.080 ± 0.01	0.142 ± 0.01	0.062 ± 0.01	0.28 ± 0.09	0.068 ± 0.01	0.152 ± 0.02	0.410 ± 0.11	0.97 ± 0.02
50-C	0.120 ± 0.02	0.225 ± 0.03	0.105 ± 0.02	0.39 ± 0.05	0.130 ± 0.02	0.229 ± 0.10	0.462 ± 0.21	1.00 ± 0.06
50-5	0.099 ± 0.02	0.162 ± 0.01	0.063 ± 0.01	0.27 ± 0.06	0.072 ± 0.01	0.145 ± 0.03	0.434 ± 0.09	0.98 ± 0.01
0-C	**0.308** ± 0.02	**0.327** ± 0.02	0.019 ± 0.03	0.17 ± 0.07	0.046 ± 0.03	0.078 ± ---	0.249 ± 0.41	1.06 ± 0.21

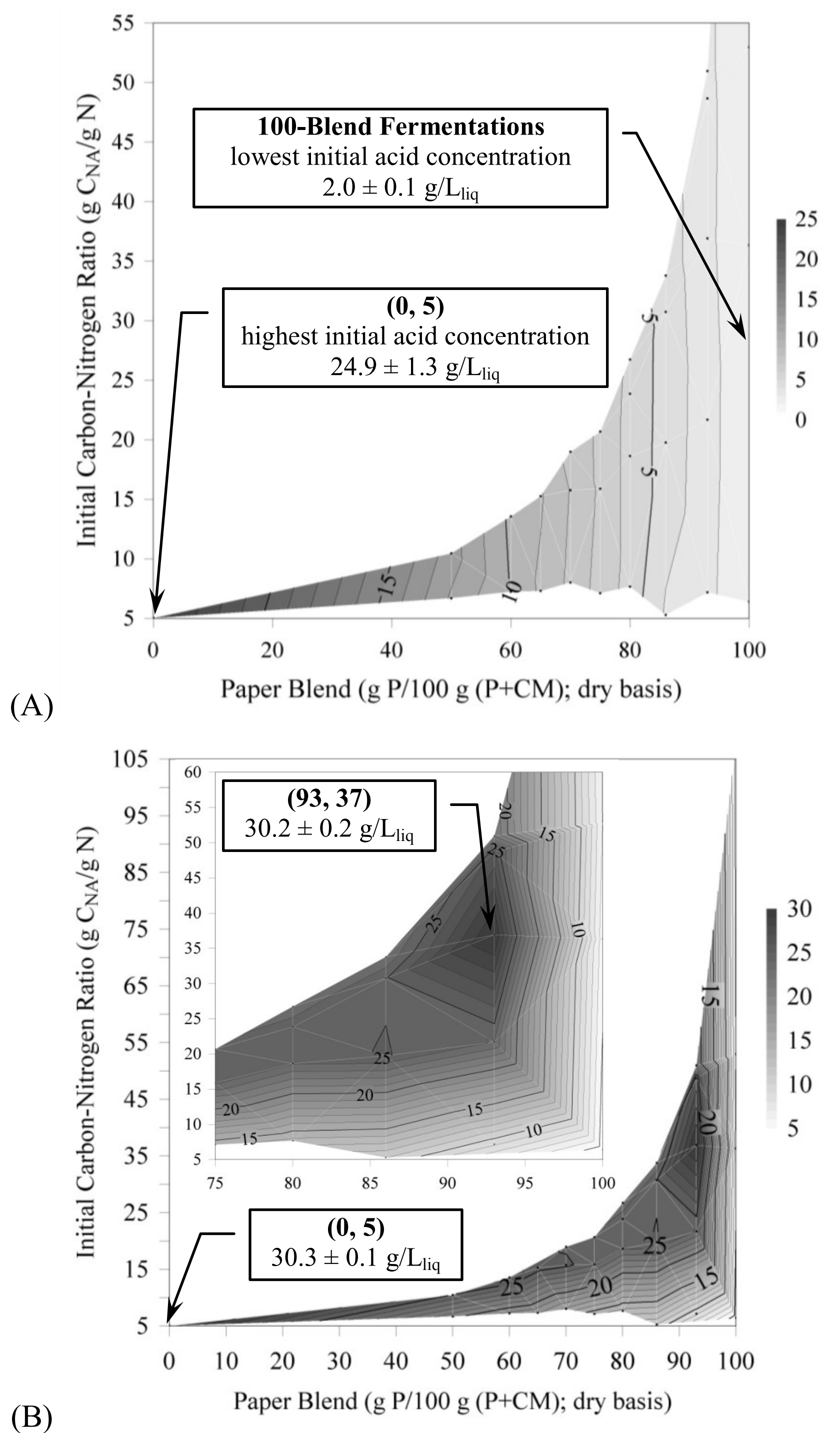


Figure 8-2. (A) Initial total carboxylic acid concentration (g/L_{liq}). (B) Final total carboxylic acid concentration (g/L_{liq}). Each contour line represents 1 g/L_{liq}. P = office paper, CM = wet chicken manure.

Some nutrient-rich wastes (e.g., wet chicken manure) have a significant acid content. For a continuous industrial fermentation, if the acid content of a feedstock is high enough, acid recovery prior to fermentation should be considered because it would reduce premature product inhibition and thereby increase the process yield. The observation that some feeds have significant carboxylic acid content inspired the definition of feed yield, exit yield, culture yield, and revised selectivity (Equations 8-8, 8-9, 8-10 and 8-12, respectively) (Smith et al., 2011).

Final acid concentration

Figure 8-2B shows the final total acid concentration contour plot. The 93-30 and 0-C fermentations had the highest final total acid concentrations, 30.2 ± 2.0 and 30.3 ± 1.3 g/L_{liq}, respectively, which were statistically similar. However, 93-30 had a higher final aceq concentration of 38.5 ± 2.2g aceq/L_{liq}. Compared to 80-C, 93-30 improved both acid and aceq concentrations by 30%.

Product spectrum

Studies have shown that the product spectrum depends on temperature, retention time, bacterial species, substrate composition (e.g., percent lignin, hexose, pentose), C/N ratio, buffer, and pH (Fu, 2007; Liu et al., 2008; Parawira et al., 2004; Playne, 1980). In this study, product spectrum was not a function of the paper blend. Figure 8-3A shows the product spectrum as a function of C/N ratio. For C/N ratios below 20 g C_{NA}/g N, the product spectrum is a strong function of C/N ratio and favors acetic acid. Above a C/N ratio of 20 g C_{NA}/g N, the proportion of acetic acid levels off and the proportion of propionic acid gradually increases. The product spectrum is relatively constant in the region of 20–40 g C_{NA}/g N, which has the highest yields; thus, C/N ratio has a limited capacity to control product spectrum. Based on Figure 8-3A, it is possible that product spectrum could be used as an indicator of nutritional circumstance, but more research is needed to test this hypothesis (Cline et al., 1958).

From Figure 8-3B, it is clear that the product spectrum is a function of pH. Acidic pH favors high-molecular-weight carboxylic acids; whereas, basic pH favors

low-molecular-weight carboxylic acids. In this study, C/N ratio and pH are related because urea was added. Low C/N-ratio fermentations, especially those supplemented with urea, correspond to basic pH and vice versa.

8.3.2. Yield

Feed yield (Equation 8-8 increased with increasing percentage of wet manure (Table 8-5) because of its inherent acid content; thus, 0-C had the highest feed yield (0.308 ± 0.02 g acid/g $NAVS_{initial}$). Figure 8-4A shows the exit yield contour plot. Fermentation 0-C also had the highest exit yield (Equation 8-9) (0.327 ± 0.02 g acid/g $NAVS_{initial}$) because its feed yield was very high. Fermentation 93-30 had the second highest exit yield (0.239 ± 0.03 g acid/g $NAVS_{initial}$), which was 21% greater than 80-C.

Figure 4B shows that Fermentation 93-30 had the highest culture yield (Equation 8-10) and aceq culture yield, 0.213 ± 0.02, and 0.273 ± 0.03 g acid/g $NAVS_{initial}$, respectively; thus, 93-30 *produced* more carboxylic acids than any other fermentation. Compared to 80-C, this was an improvement of 44% and 42%, respectively.

8.3.3. Productivity

Figure 8-4C shows the contour plot of total acid productivity. The 93-30 fermentation had the highest total acid productivity (0.84 ± 0.06 g $acid_{produced}/(L_{liq}\cdot d)$), which was a 53% improvement over 80-C. Table 8-4 shows that 93-30 started with a lower acid concentration than 80-C (3.2 vs. 5.8 g/L, respectively) yet had a much higher final acid concentration (30.2 vs. 23.3 g/L, respectively).

8.3.4. Conversion

Figure 8-4D shows the conversion contour plot. Fermentation 93-30 had the greatest conversion (0.429 ± 0.05 g $NAVS_{consumed}$/g $NAVS_{initial}$), which represents a 70% improvement verses 80-C. The dramatic improvement in conversion supports the hypothesis that proper nutrition promotes synthesis of enzymes that catalyze biomass hydrolysis and digestion.

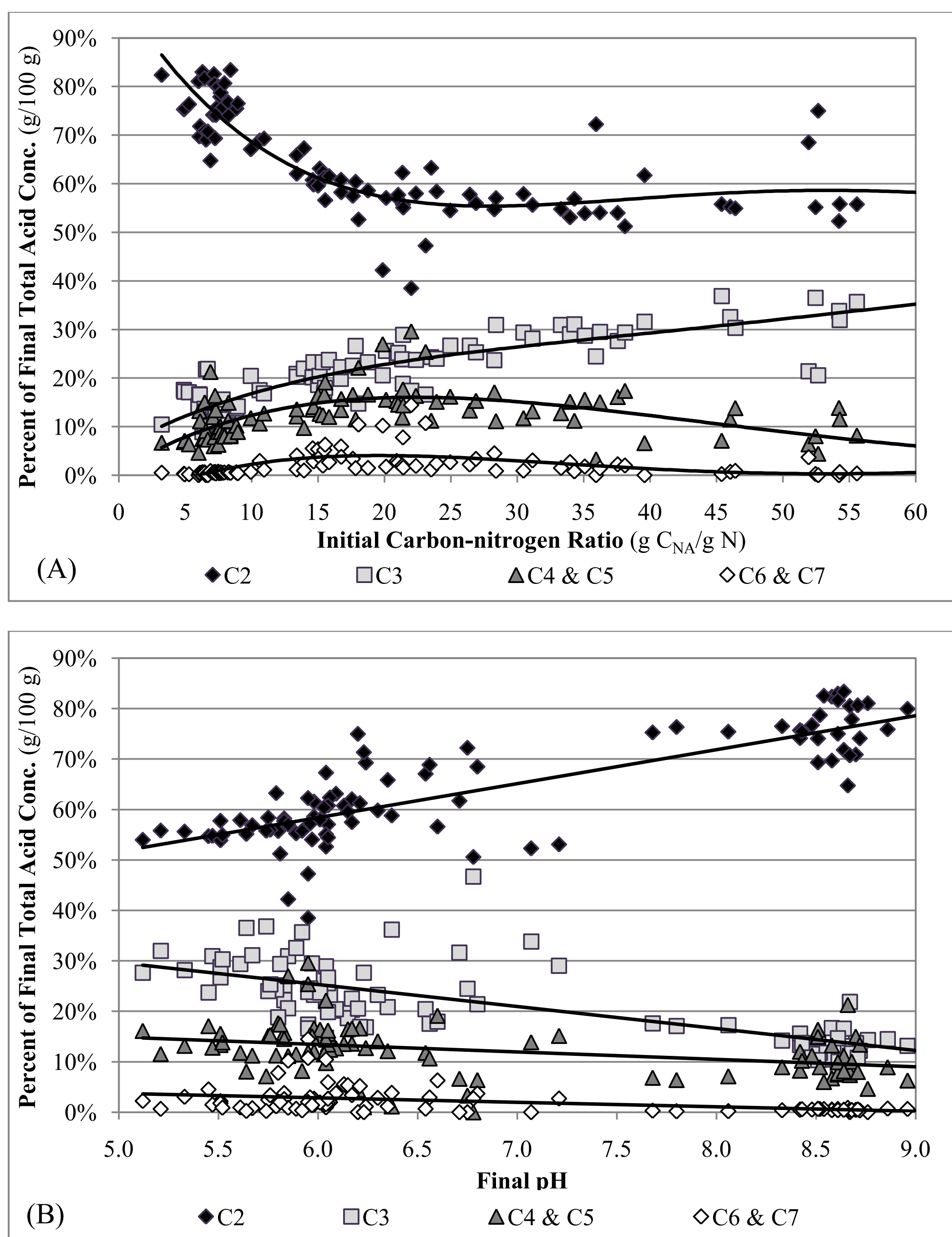


Figure 8-3. (A) Final carboxylic acid product spectrum as a function of initial C/N Ratio. Fifth-order polynomials were used as trend lines. (B) Final carboxylic acid product spectrum as a function of final pH.

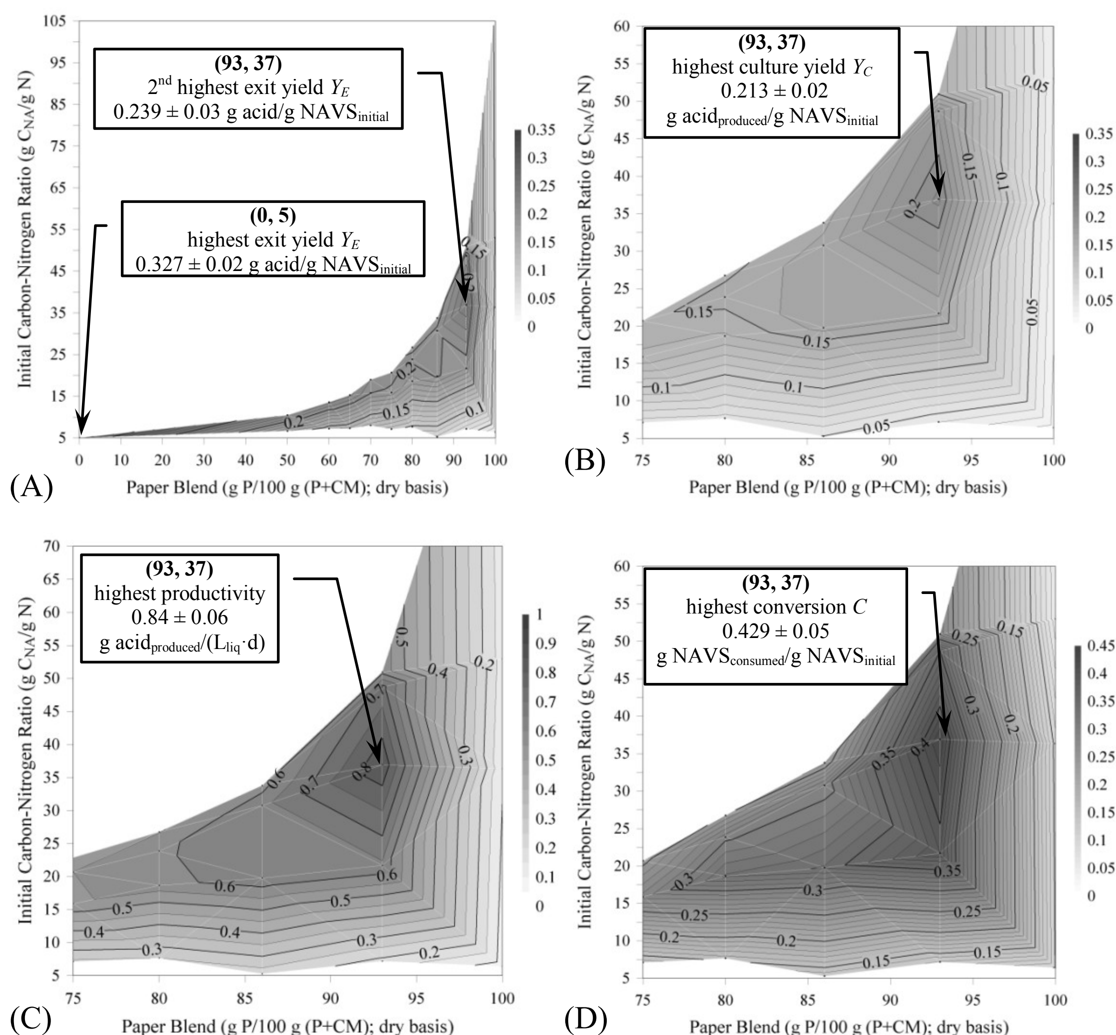


Figure 8-4. (A) Exit yield Y_E (g acid$_{produced}$/g NAVS$_{initial}$). Each contour line is 0.01 g acid$_{produced}$/g NAVS$_{initial}$. (B) Culture yield Y_C (g acid$_{produced}$/g NAVS$_{initial}$). Each contour line is 0.01 g acid$_{produced}$/g NAVS$_{initial}$. (C) Total acid productivity P (g acid$_{produced}$/(L_{liq}·d)). Each contour line is 0.05 g acid$_{produced}$/(L_{liq}·d). (D) Conversion C (g NAVS$_{consumed}$/g NAVS$_{initial}$). Each contour line is 0.01 g NAVS$_{consumed}$/g NAVS$_{initial}$. P = office paper, CM = wet chicken manure.

8.3.5. Carbon quality

Chicken manure is rich in nutrients, but has little carbohydrate and is partially digested. Office paper is almost pure polysaccharide, which provides quality carbohydrates for microbial growth and metabolism, but virtually no nutrients. The dramatic increase in productivity, culture yield, and conversion of Fermentation 93-30 can be attributed to an increase in the carbon quality, and available nitrogen. Moving from low- to high-paper blend along a constant C/N ratio line, the carbon quality increases, which increases productivity until non-nitrogen nutrients become limiting (100% paper). Unless nutrients are limiting, productivity and culture yield are directly related to the available carbohydrate concentration, not just the NAVS concentration; thus, not all NAVSs are equally good reactants. In this study, 93% carbohydrate component was the optimal blend; however, the true optimum could be a few percentage points higher or lower because the resolution in the blend was coarse.

8.3.6. Ammonia nitrogen

In this experiment, the C/N ratio was adjusted using urea; thus, along a constant C/N ratio line, the proportion of ammonia nitrogen to total nitrogen was not constant. Several studies have reported that rumen bacteria prefer high proportions of ammonia-nitrogen (50–70%) for cell synthesis (Bryant and Robinson, 1963; Hespell and Bryant, 1979; Pilgrim et al., 1970). In addition to the improved carbon quality, the higher proportion of ammonia nitrogen very likely increased cell growth, which facilitated 93-30 having the highest productivity, culture yield, and conversion. There are not enough data to decouple the contribution of improved carbon quality and increased ammonia concentration.

8.3.7. Selectivity

For the conversion of hexose to carboxylic acids, the stoichiometry and pathways are known (Bannink et al., 2006; Caspi et al., 2008; Czerkawski, 1978; Hungate, 1966; Murphy et al., 1982; Nevel and Demeyer, 1979; Offner and Sauvant, 2006; Wallnofer et

al., 1966; Wolin, 1960). Based on the literature, the theoretical maximum selectivity depends on the spectrum of acids produced. For typical carboxylic acid mixtures, assuming the only other products are H_2, CO_2, and H_2O (just metabolism; no cell growth), the theoretical maximum selectivity from sugar is 0.64–0.70 g $acid_{produced}$/g $hexose_{consumed}$. Because the NAVS are primarily sugar polymers (i.e., hexan, xylan), which lack the water of hydrolysis compared to sugars (hexose, xylose), the theoretical maximum selectivity is 0.71–0.77 g acid/g $NAVS_{consumed}$. In this paper, an average theoretical selectivity of 0.74 g $acid_{produced}$/g NAVS will be referenced. Deviations from the theoretical maximum can be attributed to (1) cell growth, (2) formation of undesired and/or unmeasured products (e.g., ethanol, acetone, lactic acid), (3) loss of acid to methane formation and/or bacterial lipid synthesis (Hespell and Bryant, 1979), (4) metabolism of other substrates that may be less efficiently converted to acid (i.e., not all reactant is hexan), and (5) experimental error. If maximum cell growth (Y_{ATP}, max = 30 g cell dry matter/mol ATP produced (Hespell and Bryant, 1979)) were achieved, which is unlikely, selectivity would be ~0.57 g $acid_{produced}$/g $NAVS_{consumed}$; thus, cell growth does not fully account for the discrepancy between theoretical and measured selectivity (Nolan, 1999). Additional hydrogen production has been hypothesized as a possible explanation; however, this is not reasonable because: (1) for a typical product spectrum, the theoretical maximum hydrogen selectivity is 0.02–0.04 g hydrogen/g $NAVS_{consumed}$, which is overstated because it is assumed that all hydrogen ions and free electrons combine to form hydrogen gas, and (2) cell growth consumed hydrogen (Nolan, 1999). Methane formation is unlikely because inhibitors were added. The extent to which acids are assimilated into lipids is unknown. The lipid content of cells is 0.05–0.20 g lipid/g dry cell matter; thus, acid assimilation into lipids *could* result in some unaccounted $NAVS_{consumed}$. Explanations 4 and 5 are also reasonable. Therefore, more studies are required to refine experimental technique and metabolic model such that selectivity closure (mass sum of products/mass sum of reactants consumed) approximates unity.

Figure 8-5A shows selectivity. Fermentation 86-C had the highest selectivity (0.663 ± 0.13 g $acid_{produced}$/g $NAVS_{consumed}$), which is statistically similar to more than

half the data set. The highest selectivities *do not* coincide with the highest exit or culture yields. Figure 8-5B shows selectivity approaches the literature-based stochiometric limit (0.74 g $acid_{produced}$/g $NAVS_{consumed}$) as C/N ratio increases (i.e., decreasing proportion of nitrogen). Because less nitrogen is available, less carbon is consumed for cell and enzyme synthesis; thus, *efficiency* towards carboxylic acids (i.e., selectivity) increases.

To illustrate the effect of paper blend on selectivity, asymptotic trend lines are fit to three data subsets (Figure 8-5B). At the same C/N ratio, lower paper blends (< 75 g P/100 g (P + CM)) had higher selectivities than higher paper blends (> 90 g P/100 g (P + CM)); however, the higher paper blends (except for the 100 blends) had higher culture yields and productivities than the lower paper blends. This observation is explained by variations in ammonia concentrations. To achieve the same C/N ratio, the higher paper blends were supplemented with more urea – which is converted to ammonia and carbon dioxide in solution – than the lower paper blends (Table 8-3). Although cellulolytic bacteria can grow using organic nitrogen (i.e., amino acids), they prefer that high proportions (50–70%) of the nitrogen be from ammonia (Bergen and Yokoyama, 1977; Hespell and Bryant, 1979; Maeng and Baldwin, 1976). Therefore, in the higher paper blends, the increased concentration of ammonia (i.e., urea) increased cell growth (Al-Rabbat et al., 1971; Bryant and Robinson, 1963; Winter et al., 1964) which produces water (measurement wise, cell mass is perceived as NAVS, not a product); thus, selectivity is decreased. Cell growth and acid formation are highly coupled. Cell growth requires ATP generated from acid (i.e., VFA) formation; thus, conditions that promote cell growth also promote acid production. Although 93-30 did not have the highest selectivity, the higher urea content and improved carbon quality promoted cell growth, which resulted in high acid production; thus, 93-30 had the highest culture yield and productivity.

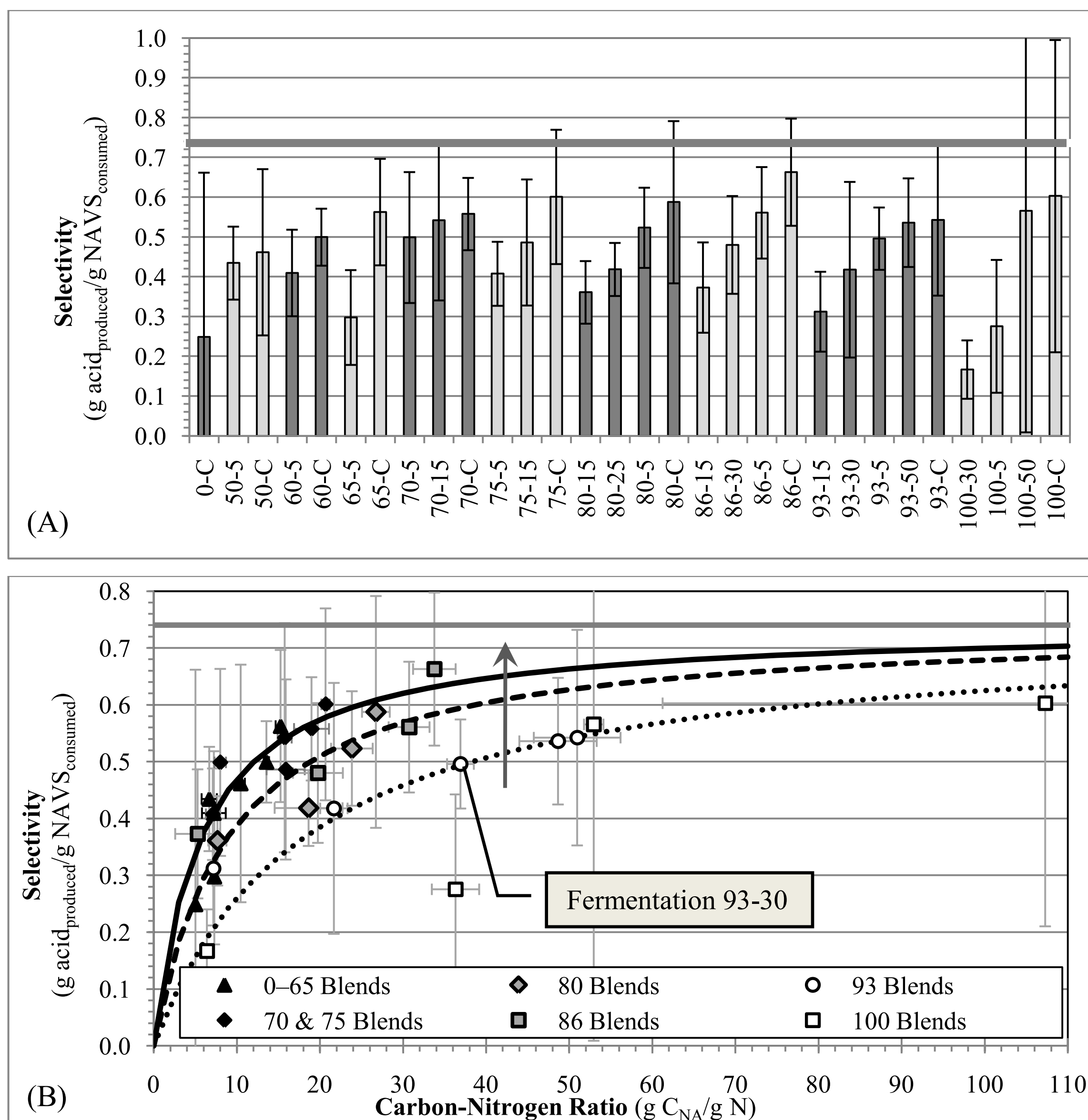


Figure 8-5. (A) Selectivity E (g $acid_{produced}$/g $NAVS_{consumed}$). (B) Selectivity E as a function of carbon-nitrogen with trend lines for different paper blend ranges. Error bars represent one standard deviation. The gray horizontal line corresponds to the average literature-based stoichiometric limit of 0.74 g $acid_{produced}$/g $NAVS_{consumed}$. Arrow indicated direction of decreasing ammonia concentration.

Based on the above observations, it is clear that selectivity is a strong function of available nutrients, especially ammonia nitrogen. The optimal scenario would have a high proportion of quality carbon (> 90%; e.g., cellulose) and with nutrient supplement to satisfy nitrogen (both organic and ammonia) and non-nitrogen requirements. Nutrient-rich wastes (e.g., manure, sewage sludge) are attractive as a nutrient source because of their low (or negative) cost. However, by attempting to satisfy *all* nutrient requirements with nutrient-rich waste, one may sacrifice yield by over-diluting the fermentation with low-quality carbon, which reduces the ammonia nitrogen concentration. To determine the economic optimum, more experimental research and economic analysis are needed to fully understand the trade-off between nutrient costs and performance.

8.3.8. Application to countercurrent fermentations

Performance contour plots (Figure 4) and optimal macro-nutrient ratios (e.g., C/N/P/S) may be used to guide operation of industrial fermentations. For example, assume a 7% chicken manure blend is optimum, but only 5% can be obtained readily. Given this feedstock constraint, the contour plots and optimal ratios can be used to calculate the amount of nitrogen or other nutrient supplements that are needed to optimize performance. Additionally, performance contour plots and nutrient ratios can be used to determine economic optima and points of diminishing return.

Although 93-30 was the best-performing batch fermentation, this may not be true for countercurrent continuous fermentations because nutrient transport, dilution gradients, and digestion gradients could alter the nutrient requirements (Smith and Holtzapple, 2010b; Smith et al., 2011). The applicability of a batch-determined optimum must be tested with continuous countercurrent fermentation. Use of nutrient transport models, as discussed by Smith and Holtzapple (2010b), may improve the application of batch studies to continuous countercurrent fermentations because the flow of nutrient species may be controlled.

8.4. Conclusions

For batch mixed-acid fermentations, the optimal C/N ratio is 20–40 g C_{NA}/g N. The amount of each feedstock, including nutrient supplements, should be engineered to satisfy optimal ratios of macro nutrients (i.e., C/N/P/S). Compared to a typical blend of 80% paper and 20% wet chicken manure (dry basis without urea), 93-30 improved the culture yield, productivity, and conversion by 53%, 44%, and 70%, respectively. These improvements have the following cost benefits: (1) decreased capital costs; (2) decreased nutrient-rich waste, which can be expensive to transport because of high moisture content and/or transportation distance; and (3) reduced downstream processing costs, which results from the improved purity of crude carboxylate salts that are less contaminated by components from the nutrient-rich waste.

9. SUMMARY

9.1. Research summary

The research presented in this dissertation began with the objective of operating a pilot fermentation at steady state. Much work was done to improve the pilot plant infrastructure, tools, and techniques needed to operate the pilot fermentation. To successfully operate a large-scale fermentation at steady state, a material balance that accounts for all solids and liquids – involved and a precise and accurate dry solid concentration method – were needed. The most difficult challenge is collecting a representative sample of solids and liquids for dry solid concentration analysis.

Pilot plant Trials 3 and 4 approximated steady state. Comparison of steady-state performance with the CPDM fermentation model (Section 4) showed that the model dramatically overestimated acid concentration (30–90% error) but more closely predicted conversion (<15% error). To determine if incorporating air in CPDM batch fermentations improved model accuracy, two sets of CPDM specific reaction rate constants were obtained – one using strict anaerobic procedures (control) and one using relaxed anaerobic procedures. Comparison of the strict and relaxed CPDM predictions showed that incorporating air in CPDM batch fermentations reduced the absolute error by >50%.

In an attempt to improve fermentation performance, urea was added during Trial 5. Qualitatively, urea addition did improve digestion because particle size dramatically decreased, which reduced the filtration performance of the screwpress and lead to the suspension of Trial 5. Trial 6 attempted to overcome filtration issues experienced during Trial 5, but the operational conditions were unreasonable for continued operation. Although Trials 5 and 6 failed to achieve steady state with urea addition, these operations provoked many questions. How much urea should be added during each transfer? What is the optimum C/N ratio for mixed-acid fermentation? To which fermentors should the urea be added? How does nitrogen move in the system? Can this behavior be controlled and/or modeled? Because the answers to these questions were

unknown, the pilot fermentation was suspended and laboratory experiments (Sections 6 and 8) were designed to investigate these questions.

The first experiment (Section 6) varied the pattern that the nutrient-rich co-substrate (i.e., chicken manure) was fed to a four-bottle countercurrent train. The broad conclusion from this experiment was that the nutrient feeding pattern does significantly impact performance. It was hypothesized that nitrogen movement was a function of physical flow of nitrogen in the solid and liquid phases *and* the reaction flux of nitrogen between these phases. Because there was insufficient data to model the latter, a segregated-nitrogen model (Section 7) was created to model just the physical flow. Comparing experimental data with the model showed that the model reasonably agrees with experimental data, physical flow dominated behavior, and the observed discrepancy with the model was caused by reaction flux between the phases.

The second laboratory experiment (Section 8) varied the blend of office paper and chicken manure, and the C/N ratio. The broad conclusions include: (1) for batch mixed-acid fermentations, the optimal C/N ratio is 20–40 g C_{NA}/g N, and (2) improved carbon quality and higher proportions of ammonia nitrogen increased cell growth, which increased productivity, culture yield, and conversion. The highest productivity, culture yield, and conversion occurred at a blend of 93 g paper/100 g (paper + chicken manure) and a nominal C/N ratio of 30 g C_{NA}/g N.

As a spin-off from countercurrent studies, to improve data analysis and reduce error, the Slope method was developed, which determines the average flowrate of a semi-continuous stream from the slope of the moving cumulative sum with respect to time. When compared to the Average and Accumulation methods (i.e., traditional methods), the Slope method did not significantly improve accuracy. However, compared to the Average method, the Slope method reduced error by more than an order of magnitude (for example data, >40% vs. <2% error). The Accumulation does not provide sufficient data to calculate error. The Slope method is an effective technique for smoothing noisy semi-continuous data, which dramatically reduces error so that statistically meaningful conclusions can more easily be made.

9.2. Key conclusions

The following subsections list key conclusions related to the topics discussed in this dissertation:

9.2.1. General

- Lignocellulose is the only renewable resource sufficiently large to replace petroleum.
- Regardless of environmental benefits (e.g., global warming), the need for energy security is sufficient motivation for the United States to aggressively develop biofuels from lignocellulose and waste.
- Mixed-acid fermentation is robust. This is not a novel conclusion, but worth stating. Throughout all fermentation work presented in this dissertation, no product was lost nor did the fermentation reaction stop as a result of contamination.

9.2.2. Pilot plant operation

- Manual paper sorting is cost prohibitive even on a small scale (e.g., 100 lb/d)
- Cleanliness, organization, and functionality are critical to safe and effective pilot plant operation.
- Solids handling and collection of a representative sample are the greatest challenges with operating a large-scale submerged fermentation
- Mixing is important but continuous vigorous mixing is not necessary. For the pilot fermentation, one hour of mixing (manual mixing combined with operating fermentor recycle loop) was sufficient to perturb less-active areas and disperse the methane inhibitor.
- Dewatering efficiency has a direct effect on performance because back mixing is reduced and less acid is lost with waste transfer solids.

9.2.3. Continuum particle distribution model (CPDM)

- CPDM batch fermentations must contain both intended (e.g., paper, chicken manure, buffer, N supplement) and unintended (e.g., air, oxygen) chemical and biochemical reactants that *are* present in the modeled system.
- The relaxed anaerobic CPDM predictions were more accurate than the strict anaerobic CPDM predictions.
- Air exposure subdued fermentation kinetics (i.e., acid production rate was reduced).
- The most significant errors are likely related to the reaction model.
- The CPDM predictions overstated acid concentration, which was an extrapolation of the batch data used to regress the empirical constants of the specific reaction rate.

9.2.4. Nitrogen in mixed-acid fermentations

- Nitrogen is a critical element that greatly influences fermentation performance.
- Maintaining optimal proportions of nutrients is critical to fermentation performance optimization.
- The segregated-nitrogen model reasonably approximates the measured nitrogen concentration profiles and captures the basic behavior of nitrogen flow in a countercurrent staged fermentation. Therefore, the segregated-nitrogen model may be used to estimate nutrient feeding strategies to achieve an optimal C/N profile, and mathematically understand the influence of input parameters on nitrogen flow.
- The discrepancies between the model and the data quantify the soluble-insoluble nitrogen reaction flux, and can be used to create a reaction-based model.
- The data in this dissertation clearly show a net reaction flux from soluble to insoluble nitrogen; however, this may not be true in general.

- The application of this model is not limited to four-stage countercurrent systems and can be adapted to model *n*-staged systems, as well as to systems with recycle loops.

9.3. Recommendations

The following subsections list recommendations for future pilot and laboratory fermentation research.

9.3.1. Pilot fermentation research

- Replace the progressive cavity pumps with more robust pumps. These pumps get damaged easily and the replacement parts are expensive.
- Purchase a filter that has a filter efficiency and rate that do not depend (or are less dependent) on particle size.
- Purchase a forced convection oven for use at the pilot plant.
- Repair leak in the hot water loop on Fermentor 1.
- Improve the temperature control on the pilot plant fermentors.
- Create/purchase a sturdy industrial-strength long-handled spoon/scoop for collecting samples from the top port of fermentors.
- Use the data from the nutrient studies to operate the pilot plant with ideal nutrient concentrations and proportions.
- Use CPDM to optimize pilot plant performance.
- Build on the solids concentration methods developed in this dissertation to improve the accuracy and precision of the methods.
- Operate at a lower solids concentration. This will increase the ability to achieve homogeneity and increase filtration rate, which will allow higher VSLRs and lower LRTs.

- Test compost pile for presence of iodine.
- Future pilot plant fermentation trials should (1) allocate time and funding to have long-term trials or (2) have equipment and personnel so that shorter LRTs can be achieved.

9.3.2. Laboratory research

- Investigate sources of error responsible for discrepancies between the CPDM predictions and reality.
- Compare acid production of strict anaerobes to facultative anaerobes.
- Determine oxygen tolerance of strict anaerobes.
- Use an oxidation reduction potential (ORP) meter to perform quantitative studies on the effect of oxygen exposure to anaerobic mixed-acid fermentation.
- Modify GC method to quantify acetone and ethanol in a sample. Otherwise, if the compounds are present as fermentation products they go unnoticed.
- Investigate cell recycling and/or "biomass reflux" on a continuous fermentation.
- Repeat the experiment presented in Section 8 with a higher resolution of data points between 85–100 g paper/100 g (paper + chicken manure) and 10–45 g C_{NA}/g N.
- With respect to Sections 6–8, perform analogous studies on other key elements such as phosphorus and potassium.
- Characterize the nitrogen reaction flux and update nitrogen model.
- Investigate parameters that regulate selectivity and create an empirical model.
- Determine if the proposed specific rate equation (Equation 4-23) improves CPDM prediction accuracy.

REFERENCES

Abdullahi, M., 2006. Characteristics of Wood ASH/OPC Concrete. *Leonardo Electronic Journal of Practices and Technologies* 8, 9–16.

Abu-Reesh, I.M., 2004. Optimal Design of Chemostats Connected in Series with Microbial Wall Growth. *Chemical and Biochemical Engineering Quarterly* 18, 167–175.

Agbogbo, F.K., 2005. Anaerobic Fermentation of Rice Straw and Chicken Manure to Carboxylic Acids Research, Ph.D. Dissertation, Texas A&M University, College Station, TX.

Agbogbo, F.K., Holtzapple, M.T., 2006. Fermentation of Rice Straw/Chicken Manure to Carboxylic Acids Using a Mixed Culture of Marine Mesophilic Micoorganisms. *Applied Biochemistry and Biotechnology* 132, 997–1014.

Agbogbo, F.K., Holtzapple, M.T., 2007. Fixed-bed fermentation of rice straw and chicken manure using a mixed culture of marine mesophilic microorganisms. *Bioresource Technology* 98, 1586–1595.

Agdag, O.N., Sponza, D.T., 2005. Co-digestion of industrial sludge with municipal solid wastes in anaerobic simulated landfilling reactors. *Process Biochemistry* 40, 1871–1879.

Aiello-Mazzarri, C., 2002. Conversion of Municipal Solid Waste To Carboxylic Acids by Anaerobic Countercurrent Fermentation Research, Ph.D. Dissertation, Texas A&M University, College Station, TX.

Aiello-Mazzarri, C., Agbogbo, F.K., Holtzapple, M.T., 2006. Conversion of municipal solid waste to carboxylic acids using a mixed culture of mesophilic microorganisms. *Bioresource Technology* 97, 47–56.

Al-Rabbat, M.F., Baldwin, R.L., Weir, W.C., 1971. Microbial Growth Dependence on Ammonia Nitrogen in the Bovine Rumen: A Quantitative Study. *Journal of Dairy Science* 54, 1162–1172.

Alley, W.M., Reilly, T.E., Franke, O.L., 1999. Sustainability of Ground-Water Resources. U.S. Government Printing Office, Denver, CO. pp. 1–86. http://pubs.usgs.gov/circ/circ1186/pdf/circ1186.pdf. Accessed: July 30, 2010.

Asif, M., Muneer, T., 2007. Energy supply, its demand and security issues for developed and emerging economies. *Renewable and Sustainable Energy Reviews* 11, 1388–1413.

Bannink, A., Kogut, J., Dijkstra, J., France, J., Kebreab, E., Van Vuuren, A.M., Tamminga, S., 2006. Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating cows. *Journal of Theoretical Biology* 238, 36–51.

Barry, T.N., Manley, T.R., Duncan, S.J., 1986. The role of condensed tannins in the nutritional value of Lotus pedunculatus for sheep. *British Journal of Nutrition* 55, 123–137.

Bergen, W.G., Yokoyama, M.T., 1977. Productive Limits to Rumen Fermentation. *Journal of Animal Science* 45, 573–584.

Bitton, G., 2005. Wastewater Microbiology. 3rd ed. John Wiley & Sons, Inc., Hoboken, NJ.

Blasig, J.D., 1991. Volatile Fatty Acid Fermentation of AFEX-Treated Newspaper and Bagasse by Rumen Microorganisms Research, M.S. Thesis, Texas A&M University, College Station, TX.

Blasig, J.D., Holtzapple, M.T., Dale, B.E., Engler, C.R., Byers, F.M., 1992. Volatile fatty acid fermentation of AFEX-treated bagasse and newspaper by rumen microorganisms. *Resources, Conservation and Recycling* 7, 95–114.

Boone, D.R., 1985. Fermentation of Anaerobic Digestion. In: P.N. Cheremisinoff, R.P. Ouellette (Eds.), Biotechnology: Applications and Research. Technomic Publishing Company, Inc., Lancaster, PA, pp. 41–51.

Bratbak, G., Dundas, I., 1984. Bacterial Dry Matter Content and Biomass Estimations. *Applied and Environmental Microbiology* 48, 755–757.

Bryant, M.P., Robinson, I.M., 1963. Apparent Incorporation of Ammonia and Amino Acid Carbon During Growth of Selected Species of Ruminal Bacteria. *Journal of Dairy Science* 46, 150–154.

Buckee, G.K., 1994. Determination of total nitrogen in barley, malt and beer by Kjeldahl procedures and the Dumas combustion method. *Journal of the Institute of Brewing* 100, 57–64.

Callaghan, F.J., Wase, D.A.J., Thayanithy, K., Forster, C.F., 2002. Continuous co-digestion of cattle slurry with fruit and vegetable wastes and chicken manure. *Biomass and Bioenergy* 22, 71–77.

Caspi, R., Foerster, H., Fulcher, C.A., Kaipa, P., Krummenacker, M., Latendresse, M., Paley, S., Rhee, S.Y., Shearer, A.G., Tissier, C., Walk, T.C., Zhang, P., Karp, P.D., 2008. The MetaCyc Database of metabolic pathways and enzymes and the BioCyc collection of Pathway/Genome Databases. *Nucleic Acids Research* 36, 623–631.

Chan, W.N., 2002. Thermophilic Anaerobic Fermentation of Waste Biomass for Producing Acetic Acid Research, Ph.D. Dissertation, Texas A&M University, College Station, TX.

Chan, W.N., Holtzapple, M.T., 2003. Conversion of Municipal Solid Wastes to Carboxylic Acids by Thermophilic Fermentation. *Applied Biochemistry and Biotechnology* 111, 93–112.

Chang, V.S., Holtzapple, M.T., 2000. Fundamental Factors Affecting Biomass Enzymatic Reactivity. *Applied Biochemstry and Biotechnology* 84–86, 5-37.

Chang, V.S., Nagwani, M., Chul-Ho, K., Holtzapple, M.T., 2001. Oxidative Lime Pretreatment of High-Lignin Biomass. *Applied Biochemstry and Biotechnology* 94, 1-28.

Chen, G., Andries, J., Luo, Z., Spliethoff, H., 2003. Biomass pyrolysis/gasification for product gas production: the overall investigation of parametric effects. *Energy Conversion and Management* 44, 1875–1884.

Clarkson, W.W., Xiao, W., 1999. Bench-scale anaerobic bioconversion of newsprint and office paper. In: J. Mata-Alvarez, F. Cecchi, A. Tilche (Eds.), 2nd International Symposium on Anaerobic Digestion of Solid Waste. IWA Publishing, Barcelona, Spain, pp. 93–100. Accessed:

Cline, J.H., Hershberger, T.V., Bentley, O.G., 1958. Utilization and/or Synthesis of Valeric Acid during the Digestion of Glucose, Starch and Cellulose by Rumen Micro-Organisms in vitro. *Journal of Animal Science* 17, 284–292.

Coleman, S., 2007. Continuous Fermentation of Food Scraps with Constant pH Control to Produce Carboxylic Acids Research, M.S. Thesis, Texas A&M University, College Station, TX.

Cox, P.M., Betts, R.A., Jones, C.D., Spall, S.A., Totterdell, I.J., 2000. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature* 408, 184–187.

Czerkawski, J.W., 1978. Reassessment of Efficiency of Synthesis of Microbial Matter in the Rumen. *Journal of Dairy Science* 61, 1261–1273.

Datta, R., 1981. Acidogenic Fermentation of Corn Stover. *Biotechnology and Bioengineering* 23, 61–77.

Davis, R.H., Ramirez, W.F., Chatterjee, A., 1990. Optimal Chemostat Cascades for Periplasmic Protein Production. *Biotechnology Progress* 6, 430–436.

Domke, S.B., 1999. Fermentation of industrial biosludge, paper fines, bagasse, and chicken manure to carboxylate salts Research, Ph.D. Dissertation, Texas A&M University, College Station, TX.

Domke, S.B., Aiello-Mazzarri, C., Holtzapple, M.T., 2004. Mixed acid fermentation of paper fines and industrial biosludge. *Bioresource Technology* 91, 41–51.

Donefer, E., Llyod, L.E., Crampton, E.W., 1960. Prediction of the Nutritive Value Index of a Forage from in vitro Rumen Fermentation Data. *Journal of Animal Science* 19, 545–552.

EIA, 2007. Annual Energy Review 2007. http://www.eia.doe.gov/emeu/aer/pdf/pages/sec11_7.pdf. Accessed: 11/13/08.

EIA, 2009. Annual Energy Review 2008, Washington, D.C. pp. 446. http://www.eia.doe.gov/aer. Accessed: June 24, 2010.

Eppley, R.W., Coatsworth, J.L., Solorzano, L., 1969. Studies of Nitrate Reductase in Marine Phytoplankton. *Limnology and Oceanography* 14, 194–205.

Etheridge, R.D., Pesti, G.M., Foster, E.H., 1998. A comparison of nitrogen values obtained utilizing the Kjeldahl nitrogen and Dumas combustion methodologies (Leco CNS 2000) on samples typical of an animal nutrition analytical laboratory. *Animal Feed Science and Technology* 73, 21–28.

Fischer, H., Polikarpov, I., Craievich, A.F., 2004. Average protein density is a molecular-weight-dependent function. *Protein Science*, 2825–2828.

Forrest, A.K., 2010. Effects of Feedstocks and Inoculum Sources on Mixed-acid and Hydrogen Fermentations Research, Ph.D. Dissertation, Texas A&M University, College Station, TX.

Forrest, A.K., Hernandez, J., Holtzapple, M.T., 2010. Effects of temperature and pretreatment conditions on mixed-acid fermentation of water hyacinths using a mixed culture of thermophilic microorganisms. *Bioresource Technology* 101, 7510–7515.

Fu, Z., 2007. Conversion of Sugarcane Bagasse to Carboxylic Acids Under Thermophillic Conditions Research, Ph.D. Dissertation, Texas A&M University, College Station, TX.

Fu, Z., Holtzapple, M., 2009. Fermentation of Sugarcane Bagasse and Chicken Manure to Calcium Carboxylates under Thermophilic Conditions. *Applied Biochemistry and Biotechnology* 162, 561-578.

Fu, Z., Holtzapple, M.T., 2010. Anaerobic mixed-culture fermentation of aqueous ammonia-treated sugarcane bagasse in consolidated bioprocessing. *Biotechnology and Bioengineering* 106, 216–227.

Fu, Z., Holtzapple, M.T., 2010b. Consolidated bioprocessing of sugarcane bagasse and chicken manure to ammonium carboxylates by a mixed culture of marine microorganisms. *Bioresource Technology* 101, 2825–2836.

Givens, D.I., Rulquin, H., 2004. Utilisation by ruminants of nitrogen compounds in silage-based diets. *Animal Feed Science and Technology* 114, 1–18.

Gleick, P.H., 1998. Water in Crisis: Paths to Sustainable Water Use. *Ecological Applications* 8, 571–579.

Golub, K.W., Smith, A.D., Hollister, E.B., Gentry, T.J., Holtzapple, M.T., 2011. Investigation of Intermittent Air Exposure on Four-Stage and One-Stage Anaerobic Semi-Continuous Mixed-Acid Fermentations. *Bioresource Technology* In Press.

Granda, C., Holtzapple, M., Luce, G., Searcy, K., Mamrosh, D., 2009. Carboxylate Platform: The MixAlco Process Part 2: Process Economics. *Applied Biochemistry and Biotechnology* 156, 107–124.

Heo, N.H., Park, S.C., Kang, H., 2004. Effects of mixture ratio and hydraulic retention time on single-stage anaerobic co-digestion of food waste and waste activated sludge. *Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances & Environmental Engineering* 39, 1739–1756.

Hespell, R.B., Bryant, M.P., 1979. Efficiency of Rumen Microbial Growth: Influence of some Theoretical and Experimental Factors on YATP. *Journal of Animal Science* 49, 1640–1659.

Holtzapple, M., Granda, C., 2009. Carboxylate Platform: The MixAlco Process Part 1: Comparison of Three Biomass Conversion Platforms. *Applied Biochemistry and Biotechnology* 156, 95–106.

Holtzapple, M.T., 2003. Lignin. 2nd ed. In: B. Caballero, L.C. Trugo, P.M. Finglas (Eds.), Encyclopedia of Food Science, Food Technology, and Nutrition. Academic Press, London, pp. 3535–3542.

Holtzapple, M.T., Davidson, R.R., Nagwani, M., 1997. Calcium Hydroxide Pretreatment of Biomass. Patent # 5693296, Texas A&M University, College Station, TX, United States of America

Holtzapple, M.T., Davison, R.R., Ross, M.K., Aldrett-Lee, S., Nagwani, M., Lee, C.-M., Lee, C., Adelson, S., Kaar, W., Gaskin, D., Chang, V.S., Loescher, M.E., 1999. Biomass Conversion to Mixed Alcohol Fuels Using the MixAlco Process. *Applied Biochemistry and Biotechnology* 77–79, 23.

Houghton, J., 2005. Global warming. *Reports on Progress in Physics* 68, 1343.

Huber, G.W., Iborra, S., Corma, A., 2006. Synthesis of Transportation Fuels from Biomass: Chemistry, Catalysts, and Engineering. *Chemical Reviews* 106, 4044–4098.

Hungate, R.E., 1966. The Rumen and Its Microbes. Academic Press, New York, NY.

Hurwitz, S., 1964. Estimation of New Phosphorus Utilization by the "Slope" Method. *Journal of Nutrition* 84, 83–92.

Jacobson, M.Z., 2008. On the causal link between carbon dioxide and air pollution mortality. *Geophysical Research Letters* 35, 1–5.

Jenkins, B.M., Baxter, L.L., Miles, T.R., 1998. Combustion properties of biomass. *Fuel Processing Technology* 54, 17–46.

Jung, H.-J.G., Fahey, G.C., Merchen, N.R., 1983. Effects of ruminant digestion and metabolism on phenolic monomers of forages. *British Journal of Nutrition* 50, 637–651.

Kayhanian, M., 1994. Performance of a high-solids anaerobic digestion process under various ammonia concentrations. *Journal of Chemical Technology & Biotechnology* 59, 349–352.

Kayhanian, M., Rich, D., 1995. Pilot-scale high solids thermophilic anaerobic digestion of municipal solid waste with an emphasis on nutrient requirements. *Biomass and Bioenergy* 8, 433–444.

Kayhanian, M., Tchobanglous, G., 1992. Computation of C/N Ratios for Various Organic Fractions BioCycle, pp. 58–60. Accessed: Dec. 10, 2009.

Kim, D.-H., Kim, S.-H., Han, S.-K., Shin, H.-S., 2006. Performance of Pilot-Scale H_2 Fermentor: C/N Ratio Effect and Alkaline Shock Treatment. *Journal of Environmental Engineering and Management* 16, 327–331.

Kim, H.-W., Han, S.-K., Shin, H.-S., 2003. The optimisation of food waste addition as a co-substrate in anaerobic digestion of sewage sludge. *Waste Management Research* 21, 515–526.

Kim, S.-H., Han, S.-K., Shin, H.-S., 2004. Feasibility of biohydrogen production by anaerobic co-digestion of food waste and sewage sludge. *International Journal of Hydrogen Energy* 29, 1607–1616.

Kim, S., Holtzapple, M.T., 2006. Delignification kinetics of corn stover in lime pretreatment. *Bioresource Technology* 97, 778-785.

Kondo, T., Ohshita, T., Kyuma, T., Touno, E., Murai, M., 1999. Characterization of soluble lignin released from alfalfa by sheep digestion. *Animal Feed Science and Technology* 80, 321–328.

Kumar, A., Sokhansanj, S., 2007. Switchgrass (*Panicum vigratum, L.)* delivery to a biorefinery using integrated biomass supply analysis and logistics (IBSAL) model. *Bioresource Technology* 98, 1033–1044.

Kunz, G.J., Leeuw, G.d., 1993. Inversion of lidar signals with the slope method. *Applied Optics* 32, 3249–3256.

Laney, K., 2006. Biofuels: Promises and Constraints. International Policy Council, Washington, D.C. http://www.agritrade.org/Publications/DiscussionPapers/IPC_Biofuels_Promises%20and%20Constraints.pdf. Accessed: July 28, 2010.

Lee, C.-M., 1993. Volatile Fatty Acid Fermentation of Lime-Treated Bagasse by Rumen Microorganisms Research, M.S. Thesis, Texas A&M University, College Station, TX.

Leng, R.A., Nolan, J.V., 1984. Nitrogen Metabolism in the Rumen. *J. Dairy Sci.* 67, 1072–1089.

Lin, C.Y., Lay, C.H., 2005. A nutrient formulation for fermentative hydrogen production using anaerobic sewage sludge microflora. *International Journal of Hydrogen Energy* 30, 285–292.

Liu, X., Liu, H., Chen, Y., Du, G., Chen, J., 2008. Effects of organic matter and initial carbon-nitrogen ratio on the bioconversion of volatile fatty acids from sewage sludge. *Journal of Chemical Technology & Biotechnology* 83, 1049–1055.

Loescher, M.E., 1996. Volatile fatty acid fermentation of biomass and kinetic modeling using the CPDM method Research, Ph.D. Dissertation, Texas A&M University, College Station, TX.

Mader, J., 2006. The U.S. Petroleum Addiction: Is it Hopeless? , University of Michigan, Ann Arbor, MI. http://www.engin.umich.edu/dept/che/research/schwank/people/Papers/USPetrol_Hopeless.pdf. Accessed: June 8, 2010.

Maeng, W.J., Baldwin, R.L., 1976. Factors Influencing Rumen Microbial Growth Rates and Yields: Effect of Amino Acid Additions to a Purified Diet with Nitrogen from Urea. *J. Dairy Sci.* 59, 648–655.

Mahmudi, H., Flynn, P., 2006. Rail vs truck transport of biomass. *Applied Biochemistry and Biotechnology* 129, 88–103.

Marchaim, U., 1992. Biogas processes for sustainable development FAO Agricultural Services Bulletin No. 95. Food and Agriculture Organization of the United Nations, Rome, Italy. http://www.fao.org/docrep/t0541e/T0541E00.htm#Contents. Accessed:

Matei, C.H., Playne, M.J., 1984. Production of volatile fatty acids from bagasse by rumen bacteria. *Applied Microbiology and Biotechnology* 20, 170–175.

McGeehan, S.L., Naylor, D.V., 1988. Automated instrumental analysis of carbon and nitrogen in plant and soil samples. *Communications in Soil Science and Plant Analysis* 19, 493–505.

Milbrandt, A., 2005. A Geographic Perspective on the Current Biomass Resource Availability in the United States. NREL, Golden, CO. pp. 1–70. http://www.nrel.gov/docs/fy06osti/39181.pdf. Accessed: 8/16/2010.

Misra, M.K., Ragland, K.W., Baker, A.J., 1993. Wood Ash Composition as a Function of Furnace Temperature. *Biomass and Bioenergy* 4, 103–116.

Mitchell, J.F.B., Manabe, S., Meleshko, V., Tokioka, T., 1990. Equilibrium Climate Change - and its Implications for the Future. In: J.T. Houghton, G.J. Jenkins, J.J. Ephraums (Eds.), Climate Change: The IPCC Scientific Assessment. Cambridge University Press, Cambridge, UK, pp. 131–172.

Moody, A.G., 2006. Pilot-scale Fermentation of Office Paper and Chicken Manure to Carboxylic Acids Research, M.S. Thesis, Texas A&M University, College Station, TX.

Murphy, M.R., Baldwin, R.L., Koong, L.J., 1982. Estimation of Stoichiometric Parameters for Rumen Fermentation of Roughage and Concentrate Diets. *Journal of Animal Science* 55, 411–421.

Nevel, C.J., Demeyer, D.I., 1979. Stoichiometry of carbohydrate fermentation and microbial growth efficiency in a continous culture of mixed rumen bacteria. *Applied Microbiology and Biotechnology* 7, 111–120.

Nolan, J.V., 1999. Stoichiometry of Rumen Fermentation and Gas Production. In: S.M. Howden, P.J. Reyenga (Eds.), Meeting the Kyoto Target: implications for the Australian livestock industries. Bureau of Rural Sciences Australia, Canberra, Australia, pp. 21–28.

Offner, A., Sauvant, D., 2006. Thermodynamic modeling of ruminal fermentations. *Animal Research* 55, 343–365.

Oztekin, R., Kapdan, I.K., Kargi, F., Argun, H., 2008. Optimization of media composition for hydrogen gas production from hydrolyzed wheat starch by dark fermentation. *International Journal of Hydrogen Energy* 33, 4083–4090.

Painter, H.A., 1970. A review of literature on inorganic nitrogen metabolism in microorganisms. *Water Research* 4, 393–450.

Parawira, W., Murto, M., Read, J.S., Mattiasson, B., 2004. Volatile fatty acid production during anaerobic mesophilic digestion of solid potato waste. *Journal of Chemical Technology & Biotechnology* 79, 673–677.

Parry, I.W.H., Darmstadter, J., 2003. The Costs of U.S. Oil Dependency Resources for the Future, Washington, D.C., pp. 40. http://www.rff.org/documents/RFF-DP-03-59.pdf. Accessed: June 24, 2010.

Phillips, S., Aden, A., Jechura, J., Dayton, D., Eggeman, T., 2007. Thermochemical Ethanol via Indirect Gasification and Mixed Alcohol Synthesis of Lignocellulosic Biomass. NREL, Golden, Colorado, pp. 132. http://www.nrel.gov/docs/fy07osti/41168.pdf. Accessed: October 29, 2008.

Pilgrim, A.F., Gray, F.V., Weller, R.A., Belling, C.B., 1970. Synthesis of microbial protein from ammonia in the sheep's rumen and the proportion of dietary nitrogen converted into microbial nitrogen. *British Journal of Nutrition* 24, 589–598.

Playne, M.J., 1980. Microbial conversion of cereal straw and bran into volatile fatty acids – key intermediates in the production of liquid fuels. *Food Technology Australia* 32, 451–456.

Price, E.C., 1985. The Microbiology of Anaerobic Digestion. In: P.N. Cheremisinoff, R.P. Ouellette (Eds.), Biotechnology: Applications and Research. Technomic Publishing Company, Inc., Lancaster, PA, pp. 52–59. Accessed: Dec. 10, 2009.

Price, E.C., Cheremisinoff, P.N., 1985. Methods of Biogas Generation and Comparisons. In: P.N. Cheremisinoff, R.P. Ouellette (Eds.), Biotechnology: Applications and Research. Technomic Publishing Company, Inc., Lancaster, PA, pp. 53–67.

Qi, B.C., Wolfaardt, G.M., Aldrich, C., Lorenzen, L., 2003. Methanogenic Digestion of Lignocellulose Residues under Conditions of High-Rate Acidogenic Fermentation. *Industrial & Engineering Chemistry Research* 42, 1845–1849.

Rapaport, A., Harmond, J., Mazenc, F., 2008. Coexistence in the design of a series of two chemostats. *Nonlinear Analysis: Real World Applications* 9, 1052–1067.

Rapier, C.R., 1995. Volatile Fatty Acid Fermentation of Lime-Treated Biomass by Rumen Microorganisms Research, M.S. Thesis, Texas A&M University, College Station, TX.

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

Ross, M.K., 1998. Production of acetic acid from waste biomass Research, Ph.D. Dissertation, Texas A&M University, College Station, TX.

Rowlands, W.N., Masters, A., Maschmeyer, T., 2008. The Biorefinery--Challenges, Opportunities, and an Australian Perspective. *Bulletin of Science Technology Society* 28, 149–158.

Salameh, M.G., 2003. Can renewable and unconventional energy sources bridge the global energy gap in the 21st century? *Applied Energy* 75, 33–42.

Sanchez, S., Demain, A.L., 2002. Metabolic regulation of fermentation processes. *Enzyme and Microbial Technology* 31, 895–906.

Searcy, E., Flynn, P., Ghafoori, E., Kumar, A., 2007. The relative cost of biomass energy transport. *Applied Biochemistry and Biotechnology* 137–140, 639-652.

Sheldrick, B.H., 1986. Test of the Leco CHN-600 Determinator for soil carbon and nitrogen analysis. *Canadian Journal of Soil Science* 66, 543–545.

Sierra, R., Smith, A., Granda, C., Holtzapple, M.T., 2008. Producing Fuels and Chemicals from Lignocellulosic Biomass Chemical Engineering Progress. American Institute of Chemical Engineers, pp. 9. www.aiche.org/cep. Accessed: March 13, 2009.

Simonne, A.H., Simonne, E.H., Eitenmiller, R.R., Mills, H.A., III, C.P.C., 1997. Could the Dumas Method Replace the Kjeldahl Digestion for Nitrogen and Crude Protein Determinations in Foods? *J Sci Food Agric* 73, 39–45.

Skoog, D.A., West, D.M., Holler, F.J., 1996. Fundamentals of Analytical Chemistry. 7th ed. Saunders College Publishing.

Smith, A.D., Holtzapple, M.T., 2010a. The Slope Method: A Tool for Analyzing Semi-Continuous Data. *Applied Biochemistry and Biotechnology* Epub. 22 Sept 2010.

Smith, A.D., Holtzapple, M.T., 2010b. Investigation of Nutrient Feeding Strategies in a Countercurrent Mixed-acid Multi-Staged Fermentation: Development of Segregated-Nitrogen Model *Bioresource Technology* 101, 9700–9709.

Smith, A.D., Landoll, M., Falls, M., Holtzapple, M.T., 2010. Survey of Chemical Production from Lignocellulosic Biomass. In: K.W. Waldron (Ed.) Bioalcohol production: Biochemical conversion of lignocellulosic biomass. Woodhead

Publishing Ltd., Cambridge, UK, pp. 560.
Smith, A.D., Lockman, N.A., Holtzapple, M.T., 2011. Investigation of Nutrient Feeding Strategies in a Countercurrent Mixed-acid Multi-Staged Fermentation: Experimental Data. *Applied Biochemistry and Biotechnology* In Press.
Smith, M.R., 2007. Filling the oil supply gap. *Petroleum Review*, 24–27.
Sosnowski, P., Wieczorek, A., Ledakowicz, S., 2003. Anaerobic co-digestion of sewage sludge and organic fraction of municipal solid wastes. *Advances in Environmental Research* 7, 609–616.
Stansbury, P.F., Whitaker, A., 1984. Media for Industrial Fermentations Principles of Fermentation Technology. Pergamon Press Ltd., Elmsford, NY, pp. 74–90.
Sue, D.Y., Wasserman, K., Moricca, R.B., Casaburi, R., 1988. Metabolic acidosis during exercise in patients with chronic obstructive pulmonary disease. Use of the V-slope method for anaerobic threshold determination. *Chest* 94, 931–938.
Sun, Y., Cheng, J., 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology* 83, 1–11.
Susmel, P., Stefanon, B., 1993. Aspects of lignin degradation by rumen microorganisms. *Journal of Biotechnology* 30, 141–148.
Sweeney, R.A., 1989. Generic combustion method for determination of crude protein in feeds: Collaborative Study. *J. Assoc. Off. Anal. Chem.* 72, 770–774.
Thanakoses, P., 2002. Conversion of Bagasse and Corn Stover to Mixed Carboxylic Acids Using a Mixed Culture of Mesophilic Microorganisms Research, Ph.D. Dissertation, Texas A&M University, College Station, TX.
Thanakoses, P., Black, A.S., Holtzapple, M.T., 2003. Fermentation of Corn Stover to Carboxylic Acids. *Biotechnology and Bioengineering* 83, 191–200.
Thomas, H.A., Jr., 1937. The "Slope" Method of Evaluating the Constants of the First-Stage Biochemical Oxygen-Demand Curve. *Sewage Works Journal* 9, 425–430.
Thomson, D.J., 1997. Dependence of global temperatures on atmospheric CO2 and solar irradiance. *Proceedings of the National Academy of Sciences USA* 94, 8370–8377.
Trevelyan, W.E., 1966. A Continuous Fermentation Technique for Studying the Kinetics of Sugar Uptake by Baker's Yeast. *Biochemical Journal* 100, 556–563.
USDOE, 2009. Annual Energy Review 2008, Washington, D.C. pp. 446. http://www.eia.doe.gov/aer. Accessed: June 24, 2010.
Vitousek, P.M., 1994. Beyond Global Warming: Ecology and Global Change. *Ecology* 75, 1861–1876.
Wallnofer, P., Baldwin, R.L., Stagno, E., 1966. Conversion of C14-Labeled Substrates to Volatile Fatty Acids by the Rumen Microbiota. *Appl. Environ. Microbiol.* 14, 1004–1010.
Wentz, F.J., Ricciardulli, L., Hilburn, K., Mears, C., 2007. How Much More Rain Will Global Warming Bring? *Science* 317, 233–235.

Wikipedia_contributors, 2010. Wikipedia. www.wikipedia.org. Accessed: September 2010.
Wilson, P.W., Peterson, W.H., Fred, E.B., 1930. The Relationship between the Nitrogen

and Carbon Metabolism of *Clostridium acetobutylicum*. *Journal of Bacteriology* 19, 231–260.

Winter, K.A., Johnson, R.R., Dehority, B.A., 1964. Metabolism of Urea Nitrogen by Mixed Cultures of Rumen Bacteria Grown on Cellulose. *Journal of Dairy Science* 47, 793–797.

Wolin, M.J., 1960. A Theoretical Rumen Fermentation Balance. *Journal of Dairy Science* 43, 1452–1459.

Yen, H.-W., Brune, D.E., 2007. Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresource Technology* 98, 130–134.

Zellmer, D.L., 1997. Spreadsheet Equivalents of Least Squares Formulas. http://zimmer.csufresno.edu/~davidz/Stat/LLSTutorial/Formulas/LLSFormulas.html#SWH7th. Accessed: 3/31/2010.

APPENDIX A

DATA FOR PILOT PLANT OPERATIONS

Table A-1. Summary of transfer data and acid concentrations for Trial 3 Fermentor 1. Red indicates data that was estimated or interpolated.

Trial 3 – Fermentor 1																
Date	**Paper fed**	**Chicken manure fed**	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	**Filter liquid removed**	$M_{filter\ liquid}$
	dry lb	dry lb	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	wet lb	g moisture/ g total
5/15/07	100	25.0	700	1.830	800	1.70	0.794	8.892	0.444	26.22	38.59	1204.7	0.793	420.0	0.880
5/18/07	100	25.0	560	1.750	800	1.50	0.803	8.868	0.437	25.98	38.43	187.9	0.700	420.0	0.950
5/21/07	100	25.0	770	1.720	800	1.65	0.806	8.859	0.437	24.83	36.90	1085.9	0.700	420.0	0.950
5/24/07	100	25.0	740	1.700	800	1.55	0.808	8.852	0.437	23.73	35.09	947.9	0.700	420.0	0.950
5/31/07	100	25.0	780	1.530	800	1.55	0.826	8.801	0.437	25.71	37.85	685.5	0.710	420.0	0.950
6/5/07	100	25.0	745	1.750	800	1.65	0.803	8.868	0.437	23.93	35.11	693.8	0.700	420.0	0.950
6/7/07	100	25.0	790	1.700	800	1.60	0.808	8.852	0.437	24.75	36.20	907.9	0.700	420.0	0.950
6/11/07	100	25.0	765	1.630	800	1.50	0.810	9.852	0.437	25.68	37.44	724.4	0.700	420.0	0.950
6/14/07	100	25.0	755	1.650	800	1.50	0.813	8.837	0.437	25.02	36.39	823.8	0.700	420.0	0.950
6/18/07	100	25.0	765	1.630	800	1.55	0.815	8.831	0.437	24.35	35.35	990.1	0.760	420.0	0.950
6/21/07	100	25.0	775	1.480	800	1.50	0.832	8.785	0.437	24.60	35.44	364.7	0.750	420.0	0.950
6/25/07	100	25.0	800	1.530	800	1.50	0.818	8.820	0.430	25.42	36.54	636.6	0.750	420.0	0.950

Table A-1. Continued.

Trial 3 – Fermentor 1															
Date	**Paper fed**	**Chicken manure fed**	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	**Filter liquid**	$M_{filter\ liquid}$
	dry lb	dry lb	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	wet lb	g moisture/ g total
6/28/07	100	25.0	750	1.570	800	1.50	0.818	8.820	0.387	25.55	36.71	483.5	0.750	420.0	0.950
7/2/07	100	25.0	810	1.500	800	1.50	0.816	8.730	0.387	25.13	35.98	598.7	0.750	430.0	0.950
7/6/07	100	25.0	730	1.600	800	1.50	0.816	8.730	0.387	25.66	36.86	510.1	0.750	430.0	0.950
7/9/07	100	25.0	800	1.500	800	1.50	0.816	8.730	0.344	25.15	36.07	546.5	0.750	430.0	0.950
7/14/07	100	25.0	735	1.600	800	1.55	0.816	8.730	0.330	25.37	36.33	494.8	0.765	425.0	0.950
7/17/07	100	25.0	770	1.700	800	1.65	0.816	8.730	0.315	24.65	35.29	576.9	0.760	425.0	0.950
7/20/07	100	25.0	780	1.550	800	1.50	0.816	8.730	0.312	25.27	35.95	731.3	0.760	425.0	0.950
7/23/07	100	25.0	760	1.550	800	1.50	0.816	8.730	0.309	25.13	35.77	648.4	0.770	425.0	0.950
7/27/07	100	25.0	770	1.480	800	1.50	0.816	8.730	0.301	26.56	37.79	444.2	0.770	425.0	0.950
7/30/07	100	25.0	800	1.530	800	1.50	0.816	8.730	0.293	26.70	38.03	933.1	0.770	425.0	0.950
8/3/07	100	25.0	770	1.480	800	1.50	0.816	8.730	0.322	26.55	38.01	600.8	0.770	425.0	0.950
8/6/07	100	25.0	815	1.540	800	1.50	0.816	8.730	0.321	25.87	37.06	1033.8	0.770	425.0	0.950

*measured values represent value input in mass balance, which was determined using Figure 3-3.

Table A-2. Summary of transfer data and acid concentrations for Trial 3 Fermentor 2. Red indicates data that was estimated or interpolated.

Trial 3 – Fermentor 2													
Date	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	**Filter liquid removed**	$M_{filter\ liquid}$
	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	wet lb	g moisture/ g total
5/15/07	780	1.80	800	1.90	0.797	8.883	0.534	21.28	32.06	595.65	0.789	2061.5	0.900
5/18/07	800	1.88	800	1.90	0.797	8.883	0.508	23.63	35.41	134.61	0.700	2341.3	0.950
5/21/07	800	1.83	800	1.90	0.797	8.883	0.508	25.69	38.48	859.28	0.700	1436.9	0.850
5/24/07	770	1.95	800	2.00	0.797	8.883	0.508	21.99	32.75	549.61	0.700	1585.0	0.900
5/31/07	790	1.87	800	1.90	0.797	8.883	0.508	22.95	34.13	470.28	0.700	973.8	0.850
6/5/07	780	2.00	800	2.00	0.797	8.883	0.508	22.43	33.50	462.44	0.700	1275.9	0.850
6/7/07	780	2.05	800	2.00	0.797	8.883	0.508	22.80	33.68	897.94	0.700	1022.3	0.850
6/11/07	820	2.00	800	2.00	0.797	8.883	0.508	23.38	34.61	1207.78	0.700	1076.8	0.950
6/14/07	780	1.92	800	1.90	0.797	8.883	0.508	21.58	31.86	826.44	0.700	1249.2	0.934
6/18/07	800	1.82	800	1.90	0.797	8.883	0.508	17.66	26.07	729.36	0.740	1317.2	0.913
6/21/07	800	2.10	800	2.00	0.797	8.883	0.508	21.51	31.50	889.94	0.710	634.9	0.960
6/25/07	805	1.93	800	1.95	0.785	9.017	0.481	20.16	29.54	821.48	0.720	669.6	0.966
6/28/07	750	1.95	800	1.95	0.785	9.017	0.455	24.24	34.88	473.27	0.720	967.7	0.968
7/2/07	805	1.82	800	1.90	0.785	9.017	0.455	20.19	29.38	366.12	0.720	564.1	0.960
7/6/07	790	1.87	800	1.90	0.785	9.017	0.455	20.98	30.70	430.55	0.720	1185.6	0.960

Table A-2. Continued.

Trial 3 – Fermentor 2													
Date	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	**Filter liquid removed**	$M_{filter\ liquid}$
	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	wet lb	g moisture/ g total
7/9/07	800	2.00	800	1.90	0.785	9.017	0.428	20.30	29.56	903.45	0.715	613.1	0.960
7/14/07	780	1.83	800	1.90	0.785	9.017	0.426	22.16	32.37	74.64	0.730	1133.9	0.940
7/17/07	770	1.85	800	1.90	0.785	9.017	0.423	20.22	29.55	131.39	0.730	914.1	0.950
7/20/07	800	1.87	800	1.90	0.785	9.017	0.411	22.76	33.01	855.67	0.725	975.2	0.940
7/23/07	805	1.99	800	1.90	0.785	9.017	0.399	22.22	32.33	1037.83	0.725	1194.6	0.950
7/27/07	790	1.80	800	1.90	0.785	9.017	0.380	20.37	29.76	141.57	0.725	1023.1	0.950
7/30/07	805	1.95	800	1.90	0.785	9.017	0.361	20.41	29.84	1064.43	0.740	1320.2	0.940
8/3/07	785	1.78	800	1.90	0.785	9.017	0.405	21.40	31.12	0.00	0.740	1294.1	0.930
8/6/07	800	2.06	800	2.00	0.785	9.017	0.392	20.84	30.32	1564.68	0.740	1333.5	0.944

*measured values represent value input in mass balance, which was determined using Figure 3-3.

Table A-3. Summary of transfer data and acid concentrations for Trial 3 Fermentor 3. Red indicates data that was estimated or interpolated.

Trial 3 – Fermentor 3															
Date	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	$I_{filter\ solids}$	**Filter liquid removed**	$M_{filter\ liquid}$	**Water fed**
	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	g ash/ g total	wet lb	g moisture/ g total	gal
5/15/07	730	2.06	800	2.2	0.770	8.96	0.589	19.72	29.09	0.00	0.700	0.589	1637.80	0.870	35.60
5/18/07	800	2.2	800	2.2	0.770	8.96	0.567	19.64	29.07	134.61	0.700	0.567	824.56	0.870	93.70
5/21/07	785	2.1	800	2.2	0.770	8.96	0.567	20.32	30.10	487.61	0.700	0.567	1194.93	0.810	107.06
5/24/07	780	2.1	800	2.2	0.770	8.96	0.567	17.80	26.51	142.94	0.700	0.567	1428.24	0.810	134.50
5/31/07	790	2.15	800	2.2	0.770	8.96	0.567	18.18	26.94	265.28	0.700	0.567	834.24	0.800	80.70
6/5/07	775	2.15	800	2.2	0.770	8.96	0.567	18.36	27.19	206.61	0.700	0.567	1205.94	0.810	131.94
6/7/07	800	2.15	800	2.2	0.770	8.96	0.567	17.93	26.55	817.94	0.700	0.567	1183.60	0.810	125.87
6/11/07	800	2	800	2.2	0.770	8.96	0.567	17.32	25.69	684.44	0.700	0.567	1352.67	0.880	98.91
6/14/07	790	2.19	800	2.2	0.770	8.96	0.567	17.26	25.59	374.78	0.700	0.567	880.00	0.880	62.71
6/18/07	800	2.2	800	2.2	0.770	8.96	0.567	18.06	26.65	307.53	0.730	0.567	1066.41	0.900	77.01
6/21/07	835	2.1	800	2.2	0.770	8.96	0.567	15.07	22.18	480.29	0.710	0.567	1123.33	0.900	49.76
6/25/07	825	2.3	800	2.2	0.754	9.21	0.545	17.65	25.92	877.20	0.695	0.545	799.76	0.875	71.20
6/28/07	810	2.15	800	2.2	0.754	9.21	0.532	15.85	23.23	0.00	0.710	0.532	1401.18	0.900	89.34
7/2/07	830	2.25	800	2.2	0.750	9.10	0.532	18.91	27.79	619.65	0.710	0.532	303.13	0.900	31.80
7/6/07	820	2.15	800	2.2	0.750	9.10	0.532	16.91	25.19	139.31	0.710	0.532	1187.91	0.930	86.81

Table A-3. Continued.

Trial 3 – Fermentor 3															
Date	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	$I_{filter\ solids}$	**Filter liquid removed**	$M_{filter\ liquid}$	**Water fed**
	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	g ash/ g total	wet lb	g moisture/ g total	gal
7/9/07	815	2.16	800	2.2	0.750	9.10	0.518	17.16	25.66	631.15	0.695	0.518	934.05	0.930	64.33
7/14/07	800	2.14	800	2.2	0.750	9.10	0.509	18.27	27.32	0.00	0.695	0.509	921.49	0.930	66.57
7/17/07	800	2.18	800	2.2	0.750	9.10	0.500	15.59	23.51	0.00	0.695	0.500	764.53	0.950	68.16
7/20/07	810	2.1	800	2.2	0.750	9.10	0.484	15.80	23.68	111.47	0.695	0.484	1094.70	0.870	37.27
7/23/07	825	2.236	800	2.2	0.750	9.10	0.468	14.01	20.97	914.46	0.720	0.468	1541.29	0.926	137.88
7/27/07	810	2.05	800	2.2	0.750	9.10	0.454	16.16	24.13	0.00	0.720	0.454	859.32	0.900	16.73
7/30/07	800	2.16	800	2.2	0.750	9.10	0.440	15.20	22.12	542.27	0.725	0.440	1366.10	0.930	103.30
8/3/07	800	2.09	800	2.2	0.750	9.10	0.486	18.98	25.86	0.00	0.725	0.486	789.88	0.930	36.41
8/6/07	785	2.04	800	2.2	0.750	9.10	0.481	16.85	22.97	190.90	0.725	0.481	1779.26	0.890	79.55

Table A-4. Summary of transfer data and acid concentrations for Trial 4 Fermentor 1. Red indicates data that was estimated or interpolated.

Trial 4 – Fermentor 1																
Date	**Paper fed**	**Chicken manure fed**	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	**Filter liquid removed**	$M_{filter\ liquid}$
	dry lb	dry lb	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	wet lb	g moisture/ g total
10/12/07	100	25.0	770	1.450	800	1.5	0.825	9.199	0.270	24.67	35.51	134.1	0.550	435.0	0.950
10/16/07	100	25.0	820	1.553	800	1.5	0.825	9.199	0.274	26.77	38.81	450.1	0.532	435.0	0.950
10/19/07	100	25.0	830	1.475	800	1.5	0.825	9.199	0.321	26.94	38.96	317.5	0.532	435.0	0.950
10/23/07	100	25.0	790	1.420	800	1.5	0.825	9.199	0.325	28.40	41.10	129.7	0.580	420.0	0.960
10/26/07	100	25.0	800	1.414	800	1.5	0.825	9.199	0.294	27.98	40.62	113.3	0.477	417.0	0.963
10/30/07	100	25.0	815	1.530	800	1.5	0.815	9.199	0.270	29.24	42.86	343.8	0.500	417.0	0.950
11/2/07	100	25.0	805	1.444	800	1.5	0.815	8.840	0.270	30.35	44.20	191.3	0.490	417.0	0.960
11/6/07	100	25.0	800	1.540	800	1.5	0.815	8.820	0.227	29.70	43.35	333.4	0.495	417.0	0.954
11/9/07	100	25.0	805	1.457	800	1.5	0.815	8.832	0.270	28.69	41.94	205.3	0.492	417.0	0.958
11/13/07	100	25.0	810	1.470	800	1.5	0.831	8.800	0.270	30.72	45.01	230.8	0.486	417.0	0.957
11/16/07	100	25.0	805	1.457	800	1.5	0.831	8.830	0.270	29.06	42.69	201.6	0.498	417.0	0.954
11/20/07	150	37.5	800	1.490	800	1.5	0.831	8.810	0.270	29.08	42.92	368.1	0.495	625.5	0.954
11/27/07	100	25.0	775	1.400	800	1.5	0.831	8.810	0.270	29.12	43.06	35.1	0.495	417.0	0.955
11/30/07	100	25.0	830	1.520	800	1.5	0.820	8.810	0.270	29.07	42.45	384.7	0.497	417.0	0.955
12/4/07	100	25.0	810	1.415	800	1.5	0.820	8.810	0.270	29.40	43.03	139.2	0.497	417.0	0.955

Table A-4. Continued.

Trial 4 – Fermentor 1															
Date	**Paper fed**	**Chicken manure fed**	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	**Filter liquid**	$M_{filter\ liquid}$
	dry lb	dry lb	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	wet lb	g moisture/ g total
12/8/07	100	25.0	790	1.530	800	1.5	0.820	8.810	0.270	30.26	44.28	297.4	0.497	417.0	0.955
12/13/07	100	25.0	805	1.400	800	1.5	0.820	8.780	0.270	30.84	45.16	113.6	0.499	417.0	0.960
12/17/07	100	25.0	815	1.343	800	1.5	0.820	8.780	0.270	29.16	42.47	28.2	0.499	417.0	0.960
12/20/07	100	0.0	805	1.530	800	1.5	0.820	8.780	0.270	27.76	40.49	287.4	0.501	417.0	0.960

Table A-5. Summary of transfer data and acid concentrations for Trial 4 Fermentor 2. Red indicates data that was estimated or interpolated.

Trial 4 – Fermentor 2													
Date	**Measured* volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	**Filter liquid removed**	$M_{filter\ liquid}$
	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	wet lb	g moisture/ g total
10/12/07	790	1.90	800	1.90	0.769	9.017	0.360	21.78	31.93	97.64	0.550	811.62	0.950
10/16/07	800	1.96	800	1.90	0.769	9.017	0.360	22.80	33.57	601.04	0.555	678.76	0.950
10/19/07	775	1.88	800	1.90	0.787	8.970	0.368	22.29	32.86	204.61	0.555	421.46	0.950
10/23/07	790	1.88	800	1.90	0.787	8.970	0.362	22.47	33.08	100.99	0.650	611.58	0.960
10/26/07	800	2.03	800	1.90	0.787	8.970	0.365	22.45	33.20	366.97	0.500	500.09	0.963
10/30/07	805	1.80	800	1.90	0.787	8.970	0.310	22.70	33.70	251.63	0.575	562.67	0.963
11/2/07	775	1.90	800	1.90	0.787	8.850	0.261	23.55	34.73	131.67	0.590	478.56	0.944
11/6/07	800	1.85	800	1.90	0.787	8.840	0.260	23.17	34.29	305.61	0.590	678.62	0.955
11/9/07	775	1.87	800	1.90	0.793	8.800	0.260	22.77	33.80	124.98	0.592	498.83	0.952
11/13/07	815	1.85	800	1.90	0.823	8.850	0.260	23.08	34.41	239.00	0.583	522.11	0.960
11/16/07	765	1.92	800	1.90	0.823	8.780	0.260	21.52	32.76	145.46	0.585	499.65	0.955
11/20/07	810	1.84	800	1.80	0.794	8.784	0.260	21.40	32.06	673.79	0.580	799.52	0.956
11/27/07	785	1.84	800	1.80	0.794	8.784	0.260	21.34	31.95	156.65	0.582	602.00	0.956
11/30/07	745	1.68	800	1.70	0.800	8.790	0.260	20.74	30.80	263.06	0.582	488.97	0.956
12/4/07	810	1.77	800	1.75	0.768	8.790	0.260	18.15	27.19	250.60	0.582	400.80	0.956

Table A-5. Continued.

Trial 4 – Fermentor 2													
Date	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	**Filter liquid**	$M_{filter\ liquid}$
	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	wet lb	g moisture/ g total
12/8/07	775	1.78	800	1.75	0.800	8.790	0.260	21.58	31.96	354.14	0.582	787.69	0.956
12/13/07	770	1.68	800	1.75	0.800	8.790	0.260	21.52	31.96	0.00	0.582	501.62	0.957
12/17/07	780	1.57	800	1.75	0.800	8.790	0.260	19.64	29.12	0.00	0.582	261.59	0.957
12/20/07	820	1.60	800	1.75	0.800	8.790	0.260	19.11	28.36	102.63	0.582	645.88	0.956

Table A-6. Summary of transfer data and acid concentrations for Trial 4 Fermentor 3. Red indicates data that was estimated or interpolated.

Trial 4 – Fermentor 3															
Date	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	$I_{filter\ solids}$	**Filter liquid removed**	$M_{filter\ liquid}$	**Water fed**
	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	g ash/ g total	wet lb	g moisture/ g total	gal
10/12/07	810	2.000	800	2.2	0.767	9.226	0.410	20.43	31.03	0.00	0.550	0.396	863.80	0.950	44.55
10/16/07	770	2.084	800	2.2	0.767	9.226	0.410	16.62	25.34	164.97	0.568	0.396	823.07	0.950	76.69
10/19/07	830	2.220	800	2.2	0.767	9.16	0.425	18.61	28.19	340.51	0.568	0.390	531.07	0.950	45.98
10/23/07	795	2.199	800	2.2	0.767	9.106	0.426	15.79	25.65	0.00	0.630	0.393	669.10	0.940	68.63
10/26/07	780	2.050	800	2.2	0.767	9.106	0.419	16.52	24.98	0.00	0.565	0.389	711.87	0.940	68.56
10/30/07	815	2.200	800	2.2	0.767	9.106	0.450	16.75	25.49	275.85	0.590	0.395	447.38	0.940	42.65
11/2/07	780	1.990	800	2.2	0.763	8.953	0.307	13.30	20.26	0.00	0.617	0.322	640.53	0.952	84.47
11/6/07	760	2.050	800	1.9	0.763	9.00	0.330	16.50	25.13	346.89	0.620	0.385	655.92	0.952	125.03
11/9/07	810	1.857	800	1.9	0.785	8.99	0.330	13.37	20.47	0.00	0.629	0.370	636.51	0.935	53.11
11/13/07	805	1.803	800	1.9	0.809	9.00	0.330	14.19	21.69	43.64	0.612	0.352	404.12	0.965	22.82
11/16/07	785	1.860	800	1.9	0.809	9.00	0.330	15.13	22.97	0.00	0.613	0.352	743.36	0.959	89.16
11/20/07	790	1.920	800	1.9	0.83	9.00	0.370	15.81	23.99	599.78	0.670	0.346	978.60	0.916	119.73
11/27/07	840	1.830	800	1.8	0.779	8.9	0.370	16.13	24.54	259.89	0.630	0.340	831.48	0.920	70.33
11/30/07	820	1.815	800	1.8	0.779	8.9	0.370	12.59	19.16	302.39	0.630	0.329	843.22	0.945	86.53
12/4/07	785	1.900	800	1.85	0.779	8.9	0.370	12.78	19.40	252.80	0.630	0.332	413.06	0.945	66.50

Table A-6. Continued.

Trial 4 – Fermentor 3

Date	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	$\boldsymbol{M_{slurry}}$	**Slurry density**	$\boldsymbol{I_{slurry}}$	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$\boldsymbol{M_{filter\ solids}}$	$\boldsymbol{I_{filter\ solids}}$	**Filter liquid**	$\boldsymbol{M_{filter\ liquid}}$	**Water fed**
	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	g ash/ g total	wet lb	g moisture/ g total	gal
12/8/07	810	1.813	800	1.85	0.779	8.9	0.370	13.74	20.59	213.75	0.630	0.325	1044.92	0.945	102.31
12/13/07	810	1.850	800	1.85	0.786	8.9	0.370	12.47	18.83	0.0	0.639	0.321	653.4	0.950	68.35
12/17/07	775	1.700	800	1.85	0.786	8.9	0.370	11.92	17.94	0.0	0.639	0.324	403.9	0.950	73.43
12/20/07	800	1.790	800	1.85	0.786	8.9	0.370	9.21	13.80	0.0	0.660	0.288	318.6	0.950	27.51

Table A-7. Summary of transfer data and acid concentrations for Trial 5 Fermentor 1. Red indicates data that was estimated or interpolated.

Trial 5 – Fermentor 1

Date	**Paper fed**	**Chicken manure fed**	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	**Filter liquid removed**	$M_{filter\ liquid}$
	dry lb	dry lb	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	wet lb	g moisture/ g total
1/18/08	100	25	810	1.250	800	1.50	0.855	8.720	0.227	14.73	21.51	0.0	0.490	417.0	0.980
1/21/08	100	25	815	1.270	800	1.50	0.855	8.660	0.242	15.57	22.82	0.0	0.500	417.0	0.980
1/25/08	100	25	850	1.401	800	1.50	0.855	8.660	0.251	18.19	26.46	227.2	0.500	417.0	0.980
1/28/08	100	25	835	1.360	800	1.50	0.855	8.660	0.237	20.26	29.33	119.8	0.500	417.0	0.977
2/1/08	100	25	840	1.530	800	1.50	0.850	8.660	0.230	19.32	28.09	437.1	0.500	417.0	0.977
2/4/08	100	25	800	1.400	800	1.50	0.844	8.660	0.219	21.10	30.70	115.1	0.500	417.0	0.974
2/8/08	100	25	795	1.540	800	1.50	0.844	8.635	0.219	23.72	34.47	367.7	0.526	417.0	0.968
2/11/08	100	25	805	1.440	800	1.50	0.844	8.635	0.227	24.66	35.91	214.5	0.538	417.0	0.966
2/15/08	100	25	835	1.520	800	1.50	0.839	8.635	0.241	25.78	37.44	443.0	0.538	417.0	0.963
2/18/08	100	25	805	1.485	800	1.50	0.839	8.650	0.249	26.55	38.70	299.2	0.530	417.0	0.963
2/22/08	100	25	810	1.470	800	1.50	0.839	8.650	0.255	27.31	40.49	275.1	0.534	417.0	0.962
2/25/08	100	25	800	1.470	800	1.50	0.839	8.650	0.261	27.08	40.00	256.3	0.534	417.0	0.962
2/29/08	100	25	810	1.420	800	1.50	0.835	8.750	0.269	26.10	37.63	175.2	0.534	417.0	0.962
3/3/08	100	25	825	1.440	800	1.50	0.837	8.750	0.251	27.66	39.93	234.4	0.500	417.0	0.962
3/7/08	100	25	800	1.510	800	1.50	0.837	8.750	0.252	30.43	44.28	302.0	0.500	417.0	0.962
3/11/08	100	25	800	1.350	800	1.50	0.837	8.750	0.255	27.96	40.47	17.3	0.520	417.0	0.950

Table A-8. Summary of transfer data and acid concentrations for Trial 5 Fermentor 2. Red indicates data that was estimated or interpolated.

Trial 5 – Fermentor 2													
Date	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	**Filter liquid removed**	$M_{filter\ liquid}$
	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	wet lb	g moisture/ g total
1/18/08	780	1.59	800	1.75	0.821	8.73	0.277	17.66	26.10	0.0	0.603	333.6	0.965
1/21/08	800	1.58	800	1.75	0.821	8.73	0.272	15.52	22.97	0.0	0.603	291.9	0.965
1/25/08	835	1.70	800	1.75	0.821	8.81	0.275	18.48	27.52	317.2	0.612	144.4	0.958
1/28/08	785	1.68	800	1.75	0.818	8.81	0.288	19.90	29.49	0.0	0.612	211.6	0.958
2/1/08	795	1.75	795	1.75	0.818	8.81	0.281	20.51	30.30	594.3	0.612	398.5	0.955
2/4/08	790	1.69	795	1.75	0.818	9.53	0.271	20.12	29.84	0.0	0.631	497.8	0.953
2/8/08	805	1.73	795	1.75	0.818	8.80	0.260	21.18	31.31	489.5	0.631	709.7	0.946
2/11/08	780	1.80	800	1.75	0.812	8.80	0.261	18.97	28.15	337.0	0.638	538.4	0.946
2/15/08	825	1.73	800	1.75	0.809	8.80	0.256	20.72	30.80	697.5	0.665	446.5	0.942
2/18/08	800	1.80	800	1.75	0.809	8.80	0.267	19.63	29.82	586.9	0.665	614.6	0.942
2/22/08	800	1.80	800	1.75	0.809	8.80	0.268	21.05	32.44	527.3	0.650	515.0	0.945
2/25/08	805	1.78	800	1.75	0.809	8.80	0.259	22.08	33.84	470.5	0.650	623.7	0.945
2/29/08	800	1.78	800	1.75	0.795	8.79	0.263	21.40	31.95	329.3	0.652	428.6	0.948
3/3/08	825	1.75	800	1.75	0.796	8.79	0.263	20.33	30.08	465.6	0.652	385.7	0.948
3/7/08	800	1.75	800	1.75	0.796	8.79	0.265	18.92	28.39	462.1	0.652	650.5	0.948
3/11/08	780	1.82	800	1.75	0.796	8.79	0.266	25.02	36.09	164.9	0.652	371.4	0.935

Table A-9. Summary of transfer data and acid concentrations for Trial 5 Fermentor 3. Red indicates data that was estimated or interpolated.

Trial 5 – Fermentor 3															
Date	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	**M_{slurry}**	**Slurry density**	**I_{slurry}**	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	**$M_{filter\ solids}$**	**$I_{filter\ solids}$**	**Filter liquid**	**$M_{filter\ liquid}$**	**Water fed**
	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	g ash/ g total	wet lb	g moisture/ g total	gal
1/18/08	770	1.838	800	1.85	0.817	8.81	0.275	14.49	21.89	0.0	0.562	0.275	500.4	0.950	90.00
1/21/08	815	1.750	800	1.85	0.817	8.89	0.313	12.65	19.11	0.0	0.610	0.313	291.9	0.950	20.00
1/25/08	780	1.620	800	1.85	0.817	8.89	0.311	13.43	20.12	0.0	0.610	0.311	0.0	0.944	0.00
1/28/08	820	1.774	800	1.85	0.817	8.91	0.315	13.91	20.89	0.0	0.610	0.315	234.0	0.944	8.05
2/1/08	765	1.770	800	1.85	0.810	8.91	0.321	15.61	23.61	212.7	0.649	0.321	535.6	0.944	59.72
2/4/08	785	1.823	800	1.85	0.810	8.91	0.322	15.72	23.50	0.0	0.649	0.322	439.1	0.941	67.65
2/8/08	810	1.850	800	1.85	0.810	8.91	0.305	15.98	24.23	458.4	0.660	0.305	733.4	0.941	76.72
2/11/08	790	1.788	800	1.85	0.810	8.91	0.310	14.99	22.99	18.8	0.652	0.310	813.3	0.941	73.93
2/15/08	725	1.820	800	1.85	0.810	8.91	0.297	15.91	24.52	133.9	0.660	0.297	468.3	0.941	71.21
2/18/08	750	1.800	800	1.85	0.810	8.91	0.283	15.16	23.32	44.1	0.660	0.283	875.0	0.941	96.41
2/22/08	775	2.000	800	1.85	0.788	8.89	0.287	14.08	21.91	624.6	0.664	0.287	744.9	0.940	127.87
2/25/08	780	1.650	800	1.85	0.788	8.89	0.268	11.30	17.39	0.0	0.664	0.268	777.2	0.940	61.77
2/29/08	742	1.820	800	1.85	0.793	8.85	0.262	14.44	21.18	0.0	0.679	0.262	567.9	0.945	90.40
3/3/08	780	1.770	800	1.85	0.798	8.83	0.272	14.09	20.36	129.3	0.679	0.272	384.2	0.945	30.68
3/7/08	760	1.850	800	1.85	0.798	8.83	0.264	18.51	26.71	134.4	0.679	0.264	794.0	0.945	100.76
3/11/08	820	1.800	800	1.85	0.798	8.83	0.266	14.67	20.63	0.0	0.679	0.266	670.4	0.920	42.67

Table A-10. Summary of transfer data and acid concentrations for Trial 6 Fermentor 1. Red indicates data that was estimated or interpolated.

Trial 6 – Fermentor 1															
Date	**Paper fed**	**Chicken manure fed**	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	**Filter liquid removed**	$M_{filter\ liquid}$
	dry lb	dry lb	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	wet lb	g moisture/ g total
5/19/08	100	25	820	1.570	800	1.5	0.821	8.815	0.260	26.49		505.15	0.56	36.00	0.95
5/22/08	100	25	805	1.541	800	1.5	0.825	8.804	0.260	27.67	40.41	312.61	0.50	36.00	0.95
5/26/08	100	25	800	1.490	800	1.5	0.830	8.790	0.260	28.09	38.29	294.16	0.52	36.00	0.95
5/29/08	100	25	810	1.490	800	1.5	0.835	8.775	0.260	27.94	40.52	298.27	0.52	36.00	0.96
6/2/08	100	25	785	1.450	800	1.5	0.833	8.782	0.260	27.67	40.12	165.28	0.52	36.00	0.96
6/5/08	100	25	805	1.450	800	1.5	0.835	8.776	0.260	27.67	40.12	205.04	0.52	36.00	0.97
6/10/08	100	25	820	1.456	800	1.5	0.834	8.778	0.262	26.77	38.82	245.19	0.51	36.00	0.97
6/13/08	100	25	770	1.480	800	1.5	0.832	8.785	0.262	26.63	38.40	152.40	0.51	36.00	0.97
6/16/08	100	25	840	1.450	800	1.50	0.835	8.776	0.260	27.34	39.42	283.33	0.51	35.75	0.97
6/19/08	100	25	800	1.450	800	1.50	0.835	8.776	0.257	26.33	37.78	181.19	0.52	35.75	0.97
6/23/08	100	25	810	1.464	800	1.50	0.833	8.780	0.243	27.16	38.97	231.97	0.52	35.75	0.97
6/26/08	100	25	830	1.450	800	1.50	0.835	8.776	0.257	29.87	43.10	250.56	0.50	35.75	0.97
6/30/08	100	25	805	1.470	800	1.50	0.833	8.782	0.276	29.51	42.53	225.37	0.51	35.75	0.97
7/3/08	100	25	810	1.530	800	1.50	0.826	8.801	0.350	26.25	37.81	338.53	0.50	35.75	0.97
7/7/08	100	25	805	1.450	800	1.50	0.835	8.776	0.346	28.60	41.18	189.11	0.50	35.75	0.97
7/10/08	100	25	810	1.460	800	1.50	0.834	8.779	0.255	27.20	39.08	219.42	0.51	35.45	0.97

Table A-10. Continued.

Trial 6 – Fermentor 1

Date	Paper fed	Chicken manure fed	Measured * volume	Measured* solid conc.	Set point volume	Set point solid conc.	M_{slurry}	Slurry density	I_{slurry}	Acid conc.	Aceq conc.	Filter solids removed	$M_{filter\ solids}$	Filter liquid removed	$M_{filter\ liquid}$
	dry lb	dry lb	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	wet lb	g moisture/ g total
7/14/08	100	25	790	1.500	800	1.50	0.829	8.791	0.238	25.48	36.52	235.01	0.51	35.45	0.97
7/17/08	100	25	815	1.465	800	1.50	0.833	8.781	0.242	27.50	39.38	241.00	0.51	35.45	0.97
7/21/08	100	25	795	1.455	800	1.50	0.834	8.778	0.264	23.86	34.36	173.53	0.51	35.45	0.97
7/25/08	100	25	800	1.465	800	1.50	0.833	8.781	0.244	22.10	31.82	203.41	0.51	35.45	0.97
7/28/08	100	25	810	1.470	800	1.50	0.833	8.782	0.243	20.55	29.59	233.11	0.51	35.45	0.97
7/31/08	100	25	800	1.470	800	1.50	0.833	8.782	0.250	19.59	28.21	197.51	0.49	35.45	0.97
8/4/08	100	25	810	1.480	800	1.50	0.832	8.785	0.260	19.55	28.16	242.06	0.49	35.45	0.97
8/7/08	100	25	800	1.520	800	1.50	0.827	8.797	0.278	19.47	28.03	282.03	0.49	35.45	0.97
8/11/08	100	25	765	1.450	800	1.50	0.835	8.776	0.247	18.96	27.30	79.15	0.49	35.45	0.97
8/14/08	100	25	815	1.450	800	1.50	0.835	8.776	0.250	15.03	21.64	204.72	0.49	35.45	0.97
8/18/08	100	25	805	1.510	800	1.50	0.828	8.794	0.234	15.79	22.74	278.78	0.49	35.45	0.97
8/21/08	100	25	800	1.470	800	1.50	0.833	8.782	0.252	16.24	23.39	196.31	0.49	35.45	0.97
8/28/08	100	25	800	1.440	800	1.5	0.836	8.773	0.274	19.77	28.47	147.29	0.49	35.45	0.97
9/1/08	100	25.0	820	1.520	800	1.5	0.827	8.797	0.259	19.22	27.68	331.88	0.49	35.45	0.97
9/4/08	100	25.0	815	1.468	800	1.5	0.833	8.782	0.255	20.31	29.25	231.85	0.49	35.45	0.97
9/9/08	100	25.0	800	1.520	800	1.5	0.827	8.797	0.239	20.64	29.72	275.44	0.48	35.45	0.96
9/12/08	100	25.0	780	1.480	800	1.5	0.832	8.785	0.252	22.16	31.92	160.65	0.48	35.45	0.96

Table A-10. Continued.

Trial 6 – Fermentor 1															
Date	**Paper fed**	**Chicken manure fed**	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	**Filter liquid removed**	$M_{filter\ liquid}$
	dry lb	dry lb	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry g	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	wet lb	g moisture/ g total
9/16/08	100	25.0	810	1.550	800	1.5	0.824	8.807	0.249	20.72	29.84	352.37	0.48	35.45	0.96
9/19/08	100	25.0	800	1.450	800	1.5	0.835	8.776	0.257	19.81	28.52	164.57	0.48	35.45	0.96
9/26/08	100	25.0	740	1.530	800	1.5	0.826	8.801	0.250	20.65	29.74	149.92	0.50	34.50	0.95
9/30/08	100	25.0	780	1.470	800	1.5	0.833	8.782	0.250	19.65	28.30	246.94	0.55	34.50	0.94

Table A-11. Summary of transfer data and acid concentrations for Trial 6 Fermentor 2. Red indicates data that was estimated or interpolated.

Trial 6 – Fermentor 1													
Date	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	**Filter liquid removed**	$M_{filter\ liquid}$
	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	wet lb	g moisture/ g total
5/19/08	770	1.45	800	1.40	0.84	8.76	0.25	16.81	24.37	1354.4	0.768	666.8	0.935
5/22/08	815	1.44	800	1.40	0.84	8.77	0.25	18.19	27.03	516.3	0.550	613.7	0.960
5/26/08	790	1.63	800	1.60	0.84	8.77	0.25	19.39	28.60	381.7	0.580	647.9	0.930
5/29/08	780	1.45	800	1.45	0.84	8.78	0.25	17.06	25.16	352.5	0.600	562.8	0.950
6/2/08	790	1.42	800	1.45	0.84	8.77	0.25	17.29	25.51	88.4	0.570	659.1	0.950
6/5/08	800	1.40	800	1.45	0.84	8.76	0.25	19.11	28.18	145.4	0.580	522.7	0.960
6/10/08	790	1.55	800	1.50	0.82	8.81	0.25	17.83	26.19	356.1	0.570	429.7	0.960
6/13/08	790	1.48	800	1.50	0.83	8.78	0.25	15.03	22.07	152.9	0.583	770.5	0.970
6/16/08	800	1.41	800	1.45	0.84	8.76	0.25	14.58	21.42	252.9	0.570	295.7	0.970
6/19/08	790	1.44	800	1.45	0.84	8.77	0.24	17.89	26.14	172.2	0.570	544.7	0.970
6/23/08	790	1.40	800	1.40	0.84	8.76	0.25	15.31	22.36	250.3	0.570	504.3	0.970
6/26/08	815	1.43	800	1.40	0.84	8.77	0.26	14.89	21.86	389.3	0.560	350.7	0.972
6/30/08	765	1.43	800	1.40	0.84	8.77	0.25	14.06	20.65	213.6	0.560	540.0	0.972
7/3/08	795	1.38	800	1.40	0.84	8.75	0.26	14.68	21.50	305.8	0.545	594.5	0.969
7/7/08	795	1.35	800	1.40	0.85	8.75	0.35	16.08	23.51	96.2	0.545	508.8	0.969
7/10/08	805	1.41	800	1.40	0.84	8.76	0.24	17.66	25.78	258.1	0.530	494.1	0.971

Table A-11. Continued.

Trial 6 – Fermentor 2													
Date	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	**Filter liquid removed**	$M_{filter\ liquid}$
	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	wet lb	g moisture/ g total
7/14/08	775	1.38	800	1.40	0.84	8.75	0.23	16.41	23.93	140.7	0.530	675.6	0.971
7/17/08	800	1.37	800	1.40	0.84	8.75	0.23	14.06	20.46	196.4	0.530	470.5	0.971
7/21/08	785	1.37	800	1.40	0.84	8.75	0.24	13.00	18.72	85.1	0.530	601.9	0.971
7/25/08	805	1.34	800	1.40	0.85	8.74	0.21	12.62	18.17	114.0	0.505	584.7	0.975
7/28/08	765	1.34	800	1.40	0.85	8.74	0.22	10.77	15.51	54.7	0.505	526.1	0.975
7/31/08	825	1.42	800	1.40	0.84	8.77	0.23	10.09	14.53	287.6	0.495	579.1	0.973
8/4/08	750	1.45	800	1.40	0.84	8.77	0.23	11.53	16.61	189.1	0.495	533.0	0.973
8/7/08	810	1.35	800	1.40	0.85	8.75	0.23	10.28	14.80	223.1	0.495	651.0	0.973
8/11/08	810	1.48	800	1.40	0.83	8.79	0.24	9.54	13.73	235.3	0.495	772.4	0.973
8/14/08	775	1.35	800	1.40	0.85	8.75	0.22	10.30	14.84	63.3	0.495	459.2	0.973
8/18/08	800	1.37	800	1.40	0.84	8.75	0.22	10.32	14.86	233.3	0.495	605.7	0.976
8/21/08	800	1.37	800	1.40	0.84	8.75	0.24	11.77	16.95	149.9	0.495	577.5	0.976
8/28/08	790	1.43	800	1.40	0.84	8.77	0.25	12.99	18.71	174.3	0.495	535.9	0.976
9/1/08	785	1.43	800	1.40	0.84	8.77	0.22	12.23	17.61	343.3	0.495	524.9	0.976
9/4/08	790	1.45	800	1.40	0.83	8.78	0.22	12.98	18.70	292.3	0.495	481.8	0.976
9/9/08	770	1.38	800	1.40	0.84	8.75	0.22	12.85	18.50	185.2	0.520	644.2	0.973

Table A-11. Continued.

Trial 6 – Fermentor 2														
Date	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	**Filter liquid removed**	$M_{filter\ liquid}$
	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	wet lb	g moisture/ g total
9/12/08	815	1.37	800	1.40	0.84	8.75	0.22	10.86	15.64	160.9	0.538	715.3	0.971
9/16/08	790	1.45	800	1.40	0.83	8.78	0.21	5.57	8.02	456.3	0.538	625.7	0.971
9/19/08	795	1.39	800	1.40	0.84	8.76	0.21	10.72	15.44	150.1	0.538	550.5	0.971
9/26/08	745	1.51	800	1.40	0.83	8.79	0.21	11.83	17.03	218.7	0.633	1051.7	0.963
9/30/08	790	1.45	800	1.40	0.83	8.78	0.21	9.54	13.73	414.2	0.650	812.3	0.920

Table A-12. Summary of transfer data and acid concentrations for Trial 6 Fermentor 3. Red indicates data that was estimated or interpolated.

Trial 6 – Fermentor 3															
Date	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	$I_{filter\ solids}$	**Filter liquid**	$M_{filter\ liquid}$	**Water fed**
	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	g ash/ g total	wet lb	g moisture/ g total	gal
5/19/08	770	1.50	800	1.40	0.83	8.79	0.30	14.61	20.46	956.5	0.780	0.300	1735.	0.920	179.6
5/22/08	905	1.40	800	1.40	0.83	2.58	0.30	14.53	19.50	1877.	0.823	0.300	671.2	0.930	133.5
5/26/08	800	1.32	800	1.25	0.85	8.73	0.30	12.62	17.86	901.4	0.823	0.300	811.0	0.930	157.6
5/29/08	805	1.34	800	1.25	0.85	8.73	0.30	8.33	12.24	823.6	0.800	0.300	785.2	0.930	142.8
6/2/08	820	1.28	800	1.25	0.85	8.73	0.30	11.67	17.15	269.1	0.800	0.300	675.9	0.950	64.83
6/5/08	770	1.24	800	1.25	0.85	8.73	0.30	11.51	16.90	0.0	0.800	0.300	471.5	0.950	70.51
6/10/08	785	1.20	800	1.25	0.86	8.70	0.29	5.72	8.41	415.8	0.830	0.291	611.6	0.960	97.68
6/13/08	810	1.25	800	1.20	0.86	8.70	0.31	8.90	13.07	446.7	0.836	0.309	860.3	0.950	111.3
6/16/08	800	1.18	800	1.20	0.86	8.70	0.28	6.45	9.47	519.7	0.850	0.278	269.8	0.960	65.14
6/19/08	790	1.25	800	1.10	0.86	8.70	0.33	10.73	15.83	1044.	0.850	0.326	622.8	0.960	107.1
6/23/08	790	1.14	800	1.10	0.87	8.68	0.37	7.48	11.03	693.2	0.850	0.374	606.3	0.960	135.1
6/26/08	795	0.94	800	1.10	0.89	8.62	0.38	7.31	10.79	188.1	0.850	0.381	345.9	0.970	27.23
6/30/08	805	1.20	800	1.10	0.86	8.70	0.38	7.32	10.78	1035.	0.850	0.384	824.4	0.970	158.6
7/3/08	800	1.15	800	1.10	0.87	8.68	0.39	6.60	9.65	1130.	0.855	0.388	606.9	0.975	170.3
7/7/08	810	1.15	800	1.10	0.87	8.68	0.25	5.65	8.14	576.1	0.855	0.251	469.2	0.975	102.0
7/10/08	810	1.05	800	1.10	0.88	8.65	0.24	6.33	9.03	569.2	0.860	0.241	484.8	0.975	86.59

Table A-12. Continued.

Trial 6 – Fermentor 3															
Date	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	$I_{filter\ solids}$	**Filter liquid**	$M_{filter\ liquid}$	**Water fed**
	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	g ash/ g total	wet lb	g moisture/ g total	gal
7/14/08	775	1.20	800	1.10	0.86	8.70	0.20	5.64	8.01	533.5	0.820	0.200	803.2	0.975	166.7
7/17/08	780	1.17	800	1.10	0.87	8.69	0.30	5.30	7.49	633.9	0.820	0.303	431.3	0.975	123.0
7/21/08	810	1.12	800	1.10	0.87	8.68	0.28	5.29	7.45	282.8	0.820	0.281	650.7	0.975	90.83
7/25/08	785	1.17	800	1.10	0.87	8.69	0.27	5.26	7.42	520.1	0.840	0.272	466.2	0.975	118.2
7/28/08	800	1.08	800	1.10	0.88	8.66	0.26	5.65	7.97	0.0	0.840	0.260	667.4	0.975	73.91
7/31/08	790	1.19	800	1.10	0.86	8.70	0.26	5.56	7.79	1213.	0.840	0.260	445.6	0.975	172.4
8/4/08	810	1.08	800	1.10	0.88	8.66	0.26	6.06	8.48	423.7	0.850	0.259	907.3	0.975	127.3
8/7/08	775	1.12	800	1.10	0.87	8.67	0.27	6.29	9.06	569.6	0.850	0.266	516.4	0.975	129.0
8/11/08	825	1.07	800	1.10	0.88	8.66	0.26	5.66	8.15	673.7	0.850	0.259	821.0	0.975	126.0
8/14/08	795	1.17	800	1.10	0.87	8.69	0.24	4.76	6.85	456.1	0.850	0.245	548.3	0.975	116.1
8/18/08	795	1.12	800	1.10	0.87	8.68	0.22	5.41	7.79	760.3	0.850	0.217	567.1	0.975	135.9
8/21/08	810	1.13	800	1.10	0.87	8.68	0.29	5.38	7.75	650.4	0.850	0.295	538.1	0.975	113.3
8/28/08	795	1.10	800	1.10	0.87	8.67	0.26	5.73	8.25	474.7	0.860	0.260	643.0	0.975	118.4
9/1/08	775	1.09	800	1.10	0.87	8.67	0.16	4.53	6.53	857.5	0.860	0.165	660.8	0.975	167.3
9/4/08	795	1.10	800	1.10	0.87	8.67	0.20	6.60	9.50	904.8	0.860	0.202	617.4	0.975	152.8
9/9/08	775	1.10	800	1.10	0.87	8.67	0.23	5.61	8.08	292.4	0.860	0.235	818.1	0.975	137.0

Table A-12. Continued.

Trial 6 – Fermentor 3															
Date	**Measured * volume**	**Measured* solid conc.**	**Set point volume**	**Set point solid conc.**	M_{slurry}	**Slurry density**	I_{slurry}	**Acid conc.**	**Aceq conc.**	**Filter solids removed**	$M_{filter\ solids}$	$I_{filter\ solids}$	**Filter liquid**	$M_{filter\ liquid}$	**Water fed**
	gal	dry lb/ gal	gal	dry lb/ gal	g moisture/ g total	wet lb/ gal	g ash/g dry	g acid/L_{liq}	g aceq/L_{liq}	wet lb	g moisture/ g total	g ash/ g total	wet lb	g moisture/ g total	gal
9/12/08	815	1.09	800	1.10	0.87	8.66	0.22	5.16	7.42	456.5	0.860	0.221	588.7	0.975	90.96
9/16/08	805	1.15	800	1.10	0.87	8.68	0.22	6.73	9.69	1689.	0.860	0.225	798.9	0.975	217.9
9/19/08	810	1.06	800	1.10	0.88	8.66	0.22	5.63	8.10	221.8	0.860	0.220	579.3	0.975	69.00
9/26/08	790	1.07	800	1.10	0.88	8.66	0.22	11.00	15.84	45.0	0.860	0.222	1571.	0.975	178.9
9/30/08	800	1.13	800	1.10	0.87	8.68	0.22	6.81	9.81	654.5	0.860	0.219	1047.	0.930	153.8

APPENDIX B

ACID CONCENTRATION DATA FOR CPDM BATCH FERMENTATIONS

Table B-1. Acid concentration data for 20-g VS/$L_{liq.}$ strict batch fermentation.

	C2	**C3**	**C4**	**IC4**	**C5**	**IC5**	**C6**	**C7**	**Total**	**Aceq**	**Aceq Ratio**	**Molar**	**Avg MW**	**g C/L**	**φ**
Day	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g aceq/ L_{liq}	g aceq/ g tot acid	mol/L_{liq}	g/mol	g/L_{liq}	mols acid/ mol aceq
0	1.23	1.00	0.00	0.24	0.00	0.32	0.07	0.14	3.00	4.12	1.37	0.04	72.25	1.43	0.61
2	1.33	0.89	0.00	0.20	0.00	0.27	0.07	0.12	2.89	3.88	1.34	0.04	71.06	1.36	0.63
4	1.98	1.38	0.04	0.23	0.00	0.30	0.08	0.13	4.15	5.44	1.31	0.06	69.84	1.92	0.66
6	3.64	2.41	0.00	0.53	0.00	0.67	0.13	0.23	7.62	10.03	1.32	0.11	70.06	3.54	0.65
8	3.51	2.42	0.08	0.80	0.08	1.00	0.22	0.20	8.31	11.40	1.37	0.12	72.18	3.96	0.61
10	3.69	2.52	0.10	0.94	0.09	1.18	0.27	0.24	9.02	12.53	1.39	0.12	72.88	4.33	0.59
12	3.50	2.12	0.08	0.78	0.08	0.97	0.23	0.20	7.96	10.90	1.37	0.11	72.05	3.79	0.61
14	4.23	2.45	0.09	0.89	0.09	1.11	0.25	0.23	9.35	12.71	1.36	0.13	71.69	4.43	0.62
16	5.07	2.67	0.10	0.98	0.10	1.22	0.27	0.20	10.61	14.22	1.34	0.15	70.99	4.99	0.63
18	5.12	2.30	0.09	0.86	0.09	1.07	0.24	0.21	9.98	13.17	1.32	0.14	70.21	4.65	0.65
20	5.97	2.33	0.09	0.89	0.09	1.10	0.24	0.20	10.91	14.15	1.30	0.16	69.38	5.03	0.67
22	6.33	2.13	0.08	0.84	0.08	1.03	0.21	0.14	10.86	13.81	1.27	0.16	68.48	4.95	0.69
24	6.68	2.05	0.08	0.82	0.08	0.98	0.20	0.13	11.02	13.85	1.26	0.16	67.94	4.99	0.70
26	6.66	1.96	0.08	0.81	0.08	0.97	0.19	0.13	10.88	13.64	1.25	0.16	67.83	4.92	0.71
28	7.11	2.04	0.09	0.86	0.09	1.02	0.20	0.13	11.54	14.45	1.25	0.17	67.77	5.21	0.71
NET	5.88	1.04	0.09	0.62	0.09	0.70	0.13	-0.01	8.54	10.33		0.13		3.78	

Table B-2. Acid concentration data for 40-g VS/$L_{liq.}$ strict batch fermentation.

	C2	C3	C4	IC4	C5	IC5	C6	C7	Total	Aceq	Aceq Ratio	Molar	Avg MW	g C/L	φ
Day	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g aceq/ L_{liq}	g aceq/ g tot acid	mol/L_{liq}	g/mol	g/L_{liq}	mols acid/ mol aceq
0	1.56	1.15	0.05	0.34	0.06	0.33	0.07	0.13	3.69	5.04	1.36	0.05	71.90	1.75	0.61
2	1.99	1.47	0.06	0.32	0.06	0.31	0.07	0.12	4.40	5.84	1.33	0.06	70.48	2.06	0.64
4	2.68	1.90	0.05	0.34	0.00	0.32	0.07	0.13	5.50	7.09	1.29	0.08	69.14	2.53	0.67
6	3.64	3.00	0.00	0.43	0.05	0.58	0.09	0.10	7.89	10.24	1.30	0.11	69.37	3.64	0.67
8	4.33	3.31	0.00	0.72	0.09	1.12	0.17	0.14	9.89	13.24	1.34	0.14	70.92	4.64	0.63
10	4.63	3.36	0.06	0.89	0.11	1.40	0.25	0.20	10.91	14.87	1.36	0.15	71.85	5.18	0.61
12	5.07	3.41	0.07	0.90	0.11	1.41	0.26	0.21	11.44	15.46	1.35	0.16	71.41	5.40	0.62
14	6.90	4.47	0.08	1.17	0.15	1.81	0.32	0.24	15.15	20.32	1.34	0.21	71.02	7.12	0.63
16	5.22	3.02	0.07	0.81	0.12	1.28	0.24	0.19	10.94	14.57	1.33	0.15	70.66	5.12	0.64
18	6.06	3.17	0.07	0.86	0.12	1.34	0.25	0.18	12.05	15.85	1.31	0.17	70.02	5.60	0.65
20	6.70	3.18	0.07	0.86	0.12	1.33	0.24	0.17	12.68	16.45	1.30	0.18	69.40	5.84	0.67
22	8.47	3.62	0.08	0.99	0.14	1.52	0.28	0.20	15.31	19.63	1.28	0.22	68.84	7.01	0.68
24	8.37	3.41	0.08	0.93	0.13	1.43	0.26	0.18	14.79	18.84	1.27	0.22	68.54	6.74	0.69
26	8.51	3.30	0.07	0.91	0.13	1.37	0.24	0.16	14.70	18.60	1.26	0.22	68.22	6.68	0.70
28	8.61	3.25	0.08	0.90	0.13	1.36	0.24	0.16	14.72	18.56	1.26	0.22	68.11	6.68	0.70
NET	7.05	2.10	0.02	0.56	0.06	1.04	0.17	0.03	11.02	13.53		0.16		4.92	

Table B-3. Acid concentration data for 70-g VS/$L_{liq.}$ strict batch fermentation.

	C2	**C3**	**C4**	**IC4**	**C5**	**IC5**	**C6**	**C7**	**Total**	**Aceq**	**Aceq Ratio**	**Molar**	**Avg MW**	**g C/L**	**φ**
Day	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g aceq/ L_{liq}	g aceq/ g tot acid	mol/L_{liq}	g/mol	g/L_{liq}	mols acid/ mol aceq
0	1.95	1.29	0.07	0.45	0.08	0.31	0.07	0.12	4.34	5.81	1.34	0.06	70.99	2.04	0.63
2	2.40	2.02	0.06	0.41	0.06	0.29	0.07	0.09	5.42	7.11	1.31	0.08	69.93	2.51	0.65
4	2.89	2.37	0.08	0.43	0.08	0.32	0.07	0.11	6.36	8.29	1.30	0.09	69.59	2.94	0.66
6	4.60	4.19	0.07	0.63	0.07	0.62	0.10	0.11	10.38	13.48	1.30	0.15	69.44	4.79	0.67
8	5.74	5.37	0.09	1.07	0.10	1.51	0.22	0.19	14.29	19.28	1.35	0.20	71.33	6.74	0.62
10	5.43	4.99	0.09	1.23	0.11	1.89	0.32	0.25	14.31	19.79	1.38	0.20	72.64	6.85	0.60
12	6.16	5.43	0.11	1.44	0.13	2.31	0.42	0.36	16.37	22.83	1.40	0.22	73.12	7.87	0.59
14	6.78	5.68	0.12	1.55	0.15	2.50	0.47	0.41	17.64	24.59	1.39	0.24	73.06	8.48	0.59
16	7.79	6.13	0.13	1.68	0.17	2.85	0.60	0.56	19.93	27.84	1.40	0.27	73.19	9.60	0.59
18	7.66	5.59	0.13	1.55	0.16	2.66	0.56	0.52	18.84	26.15	1.39	0.26	72.84	9.04	0.59
20	8.61	5.83	0.14	1.62	0.18	2.78	0.59	0.53	20.27	27.89	1.38	0.28	72.37	9.67	0.60
22	8.16	5.00	0.11	1.40	0.15	2.35	0.48	0.39	18.05	24.48	1.36	0.25	71.58	8.54	0.62
24	8.62	4.88	0.11	1.36	0.15	2.27	0.47	0.38	18.24	24.47	1.34	0.26	71.03	8.57	0.63
26	9.10	4.74	0.11	1.33	0.14	2.21	0.45	0.36	18.44	24.49	1.33	0.26	70.51	8.62	0.64
28	10.13	5.05	0.11	1.42	0.15	2.34	0.48	0.36	20.04	26.44	1.32	0.29	70.19	9.33	0.65
NET	8.19	3.76	0.04	0.96	0.07	2.03	0.41	0.25	15.70	20.63		0.22		7.29	

Table B-4. Acid concentration data for 100-g VS/$L_{liq.}$ strict batch fermentation.

	C2	C3	C4	IC4	C5	IC5	C6	C7	Total	Aceq	Aceq Ratio	Molar	Avg MW	g C/L	φ
Day	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g aceq/ L_{liq}	g aceq/ g tot acid	mol/L_{liq}	g/mol	g/L_{liq}	mols acid/ mol aceq
0	2.26	1.44	0.09	0.55	0.10	0.31	0.07	0.12	4.94	6.57	1.33	0.07	70.65	2.31	0.64
2	2.84	2.65	0.08	0.48	0.09	0.30	0.07	0.10	6.61	8.65	1.31	0.09	69.83	3.06	0.66
4	3.66	3.30	0.10	0.55	0.11	0.47	0.10	0.13	8.42	11.05	1.31	0.12	69.93	3.91	0.65
6	4.40	4.38	0.10	0.56	0.09	0.84	0.16	0.20	10.73	14.28	1.33	0.15	70.63	5.02	0.64
8	5.02	4.51	0.11	0.76	0.11	1.83	0.36	0.61	13.32	18.71	1.40	0.18	73.48	6.43	0.58
10	5.85	5.43	0.13	1.08	0.15	2.41	0.45	0.78	16.29	23.16	1.42	0.22	74.22	7.93	0.57
12	5.36	4.95	0.13	1.07	0.15	2.31	0.44	0.79	15.20	21.77	1.43	0.20	74.65	7.43	0.56
14	6.34	5.60	0.14	1.21	0.17	2.60	0.50	0.93	17.50	24.96	1.43	0.24	74.40	8.53	0.57
16	5.33	4.60	0.12	1.00	0.14	2.14	0.42	0.76	14.51	20.65	1.42	0.20	74.28	7.06	0.57
18	5.95	4.84	0.12	1.04	0.15	2.24	0.44	0.78	15.56	21.97	1.41	0.21	73.82	7.54	0.58
20	5.53	4.25	0.11	0.91	0.13	1.96	0.38	0.70	13.97	19.61	1.40	0.19	73.49	6.75	0.58
22	6.97	5.04	0.13	1.10	0.16	2.38	0.47	0.85	17.09	23.89	1.40	0.23	73.23	8.23	0.59
24	7.28	5.14	0.13	1.11	0.16	2.39	0.48	0.85	17.55	24.43	1.39	0.24	72.99	8.43	0.59
26	7.62	5.27	0.14	1.15	0.17	2.46	0.48	0.84	18.13	25.15	1.39	0.25	72.80	8.69	0.59
28	8.32	5.64	0.15	1.26	0.19	2.69	0.53	0.92	19.71	27.35	1.39	0.27	72.83	9.45	0.59
NET	6.07	4.19	0.07	0.70	0.09	2.38	0.47	0.81	14.77	20.78		0.20		7.14	

Table B-5. Acid concentration data for 100-g VS/$L_{liq.}$-plus-20-g carboxylate salts/$L_{liq.}$ strict batch fermentation. The *target* initial carboxylate salt concentrations were 9 g calcium acetate/$L_{liq.}$, 9 g calcium propionate/$L_{liq.}$, and 2 g butyric acid/$L_{liq.}$.

	C2	**C3**	**C4**	**IC4**	**C5**	**IC5**	**C6**	**C7**	**Total**	**Aceq**	**Aceq Ratio**	**Molar**	**Avg MW**	**g C/L**	**φ**
Day	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g aceq/ L_{liq}	g aceq/ g tot acid	mol/L_{liq}	g/mol	g/L_{liq}	mols acid/ mol aceq
0	5.98	5.79	0.08	1.80	0.08	0.21	0.05	0.09	14.09	18.26	1.30	0.20	69.35	6.49	0.67
2	7.24	7.30	0.09	1.95	0.08	0.24	0.06	0.08	17.03	21.97	1.29	0.25	69.12	7.83	0.67
4	8.96	8.85	0.11	2.26	0.11	0.32	0.07	0.10	20.78	26.75	1.29	0.30	69.00	9.54	0.68
6	10.83	10.45	0.13	2.60	0.15	0.45	0.10	0.13	24.83	31.93	1.29	0.36	68.97	11.39	0.68
8	11.24	10.74	0.14	2.60	0.15	0.51	0.12	0.14	25.64	32.95	1.29	0.37	68.95	11.75	0.68
10	9.62	9.08	0.12	2.13	0.13	0.46	0.11	0.12	21.76	27.94	1.28	0.32	68.90	9.97	0.68
12	11.34	10.80	0.15	2.42	0.14	0.66	0.15	0.12	25.78	33.14	1.29	0.37	68.95	11.82	0.68
14	10.48	9.95	0.13	2.35	0.13	1.00	0.21	0.16	24.41	31.76	1.30	0.35	69.52	11.27	0.66
16	11.61	10.68	0.14	2.63	0.14	1.58	0.30	0.12	27.21	35.66	1.31	0.39	69.87	12.62	0.66
18	11.35	10.06	0.14	2.48	0.14	1.67	0.32	0.13	26.29	34.49	1.31	0.38	69.91	12.20	0.65
20	12.55	10.61	0.16	2.58	0.16	1.82	0.36	0.14	28.37	37.09	1.31	0.41	69.75	13.14	0.66
22	12.59	10.23	0.14	2.49	0.15	1.76	0.34	0.13	27.83	36.23	1.30	0.40	69.54	12.86	0.66
24	12.23	9.71	0.14	2.38	0.15	1.68	0.32	0.13	26.74	34.74	1.30	0.38	69.45	12.34	0.67
26	12.33	9.62	0.15	2.36	0.16	1.69	0.33	0.13	26.77	34.75	1.30	0.39	69.41	12.34	0.67
28	12.68	9.73	0.16	2.42	0.17	1.74	0.34	0.14	27.38	35.53	1.30	0.39	69.40	12.62	0.67
NET	6.70	3.94	0.08	0.62	0.09	1.53	0.29	0.05	13.29	17.27		0.19		6.13	

Table B-6. Acid concentration data for 20-g VS/$L_{liq.}$ relaxed batch fermentation.

	C2	**C3**	**C4**	**IC4**	**C5**	**IC5**	**C6**	**C7**	**Total**	**Aceq**	**Aceq Ratio**	**Molar**	**Avg MW**	**g C/L**	**φ**
Day	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g aceq/ L_{liq}	g aceq/ g tot acid	mol/L_{liq}	g/mol	g/L_{liq}	mols acid/ mol aceq
0	1.19	1.07	0.00	0.16	0.00	0.13	0.00	0.00	2.56	3.25	1.27	0.04	68.34	1.16	0.69
2	1.26	1.03	0.00	0.18	0.00	0.13	0.00	0.00	2.60	3.27	1.26	0.04	68.03	1.18	0.70
4	1.98	1.37	0.04	0.22	0.04	0.16	0.00	0.04	3.85	4.83	1.26	0.06	67.88	1.74	0.70
6	2.97	1.82	0.05	0.29	0.04	0.20	0.05	0.05	5.46	6.79	1.24	0.08	67.48	2.46	0.72
8	2.74	1.27	0.08	0.42	0.13	0.40	0.09	0.00	5.12	6.58	1.28	0.07	68.90	2.35	0.68
10	3.17	1.71	0.05	0.37	0.06	0.31	0.07	0.00	5.74	7.17	1.25	0.08	67.66	2.59	0.71
12	2.73	1.28	0.08	0.42	0.13	0.40	0.09	0.00	5.13	6.59	1.29	0.07	68.96	2.35	0.68
14	3.14	1.30	0.08	0.46	0.14	0.43	0.10	0.04	5.69	7.29	1.28	0.08	68.81	2.60	0.68
16	3.22	1.12	0.09	0.48	0.15	0.43	0.10	0.04	5.63	7.17	1.27	0.08	68.58	2.57	0.69
18	3.08	0.93	0.08	0.45	0.14	0.40	0.09	0.04	5.20	6.60	1.27	0.08	68.32	2.37	0.69
20	3.37	0.88	0.11	0.52	0.19	0.46	0.12	0.05	5.70	7.29	1.28	0.08	68.71	2.60	0.68
22	3.84	0.88	0.12	0.57	0.20	0.50	0.13	0.07	6.32	8.05	1.27	0.09	68.50	2.88	0.69
24	3.80	0.72	0.11	0.56	0.20	0.49	0.13	0.07	6.09	7.72	1.27	0.09	68.34	2.77	0.69
26	3.83	0.57	0.12	0.57	0.21	0.48	0.13	0.08	5.98	7.56	1.26	0.09	68.18	2.72	0.70
28	3.94	0.44	0.14	0.65	0.25	0.53	0.14	0.08	6.15	7.83	1.27	0.09	68.53	2.81	0.69
NET	2.75	-0.63	0.14	0.48	0.25	0.39	0.14	0.08	3.59	4.59		0.05		1.65	

Table B-7. Acid concentration data for 40-g VS/$L_{liq.}$ relaxed batch fermentation.

	C2	C3	C4	IC4	C5	IC5	C6	C7	Total	Aceq	Aceq Ratio	Molar	Avg MW	g C/L	φ
Day	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g aceq/ L_{liq}	g aceq/ g tot acid	mol/L_{liq}	g/mol	g/L_{liq}	mols acid/ mol aceq
0	1.37	1.09	0.00	0.25	0.05	0.12	0.00	0.00	2.88	3.67	1.27	0.04	68.54	1.31	0.69
2	1.55	1.28	0.00	0.23	0.05	0.12	0.00	0.00	3.22	4.07	1.26	0.05	68.17	1.46	0.70
4	2.59	2.04	0.06	0.30	0.05	0.18	0.05	0.04	5.30	6.70	1.27	0.08	68.24	2.41	0.70
6	2.79	2.00	0.00	0.27	0.00	0.21	0.00	0.00	5.27	6.49	1.23	0.08	67.07	2.36	0.73
8	3.47	2.43	0.06	0.42	0.07	0.48	0.07	0.04	7.03	9.00	1.28	0.10	68.80	3.22	0.68
10	3.59	2.39	0.07	0.50	0.08	0.64	0.10	0.06	7.43	9.67	1.30	0.11	69.52	3.43	0.66
12	4.24	2.60	0.08	0.62	0.10	0.86	0.16	0.11	8.78	11.55	1.32	0.13	70.03	4.08	0.65
14	4.60	2.67	0.09	0.67	0.11	0.90	0.17	0.12	9.33	12.23	1.31	0.13	69.87	4.33	0.66
16	5.08	2.72	0.10	0.73	0.12	0.98	0.19	0.14	10.06	13.16	1.31	0.14	69.76	4.66	0.66
18	4.53	2.24	0.09	0.62	0.10	0.82	0.16	0.12	8.68	11.27	1.30	0.13	69.43	4.00	0.67
20	4.28	1.98	0.08	0.56	0.09	0.73	0.15	0.12	7.99	10.31	1.29	0.12	69.17	3.67	0.67
22	5.59	2.38	0.10	0.70	0.12	0.89	0.18	0.13	10.08	12.90	1.28	0.15	68.74	4.61	0.68
24	5.58	2.20	0.10	0.72	0.13	0.91	0.18	0.14	9.96	12.76	1.28	0.14	68.82	4.56	0.68
26	6.05	2.19	0.12	0.81	0.15	1.01	0.20	0.16	10.69	13.73	1.28	0.16	68.90	4.90	0.68
28	5.47	1.79	0.12	0.79	0.15	0.97	0.19	0.15	9.64	12.45	1.29	0.14	69.13	4.43	0.67
NET	4.10	0.71	0.12	0.54	0.10	0.84	0.19	0.15	6.77	8.78		0.10		3.12	

Table B-8. Acid concentration data for 70-g VS/$L_{liq.}$ relaxed batch fermentation.

	C2	C3	C4	IC4	C5	IC5	C6	C7	Total	Aceq	Aceq Ratio	Molar	Avg MW	g C/L	φ
Day	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g aceq/ L_{liq}	g aceq/ g tot acid	mol/L_{liq}	g/mol	g/L_{liq}	mols acid/ mol aceq
0	2.01	1.50	0.07	0.37	0.08	0.16	0.00	0.00	4.20	5.35	1.28	0.06	68.60	1.92	0.69
2	2.34	2.06	0.07	0.51	0.07	0.17	0.04	0.00	5.26	6.80	1.29	0.08	69.18	2.42	0.67
4	3.89	3.50	0.09	0.62	0.07	0.28	0.08	0.00	8.53	10.90	1.28	0.12	68.67	3.90	0.68
6	3.53	3.34	0.07	0.48	0.07	0.40	0.07	0.00	7.96	10.25	1.29	0.12	69.01	3.65	0.68
8	4.38	3.91	0.09	0.59	0.10	0.66	0.11	0.07	9.91	12.92	1.30	0.14	69.62	4.58	0.66
10	5.35	5.11	0.13	0.88	0.13	1.41	0.24	0.14	13.38	18.05	1.35	0.19	71.30	6.31	0.62
12	5.23	3.82	0.11	0.65	0.13	1.24	0.30	0.42	11.90	16.10	1.35	0.17	71.46	5.62	0.62
14	6.63	4.70	0.14	0.84	0.16	1.65	0.39	0.57	15.09	20.50	1.36	0.21	71.67	7.15	0.62
16	6.66	4.56	0.15	0.90	0.17	1.70	0.38	0.58	15.10	20.54	1.36	0.21	71.75	7.16	0.62
18	6.97	4.59	0.16	0.93	0.18	1.72	0.38	0.56	15.49	20.98	1.35	0.22	71.52	7.32	0.62
20	6.80	4.28	0.16	0.89	0.17	1.65	0.36	0.53	14.84	20.05	1.35	0.21	71.37	7.00	0.62
22	6.65	3.95	0.15	0.84	0.16	1.53	0.34	0.48	14.09	18.91	1.34	0.20	71.04	6.63	0.63
24	7.66	4.40	0.18	1.00	0.20	1.81	0.40	0.57	16.23	21.84	1.35	0.23	71.18	7.64	0.63
26	7.03	3.83	0.17	0.96	0.19	1.71	0.38	0.54	14.81	19.98	1.35	0.21	71.33	6.98	0.62
28	7.93	4.09	0.21	1.14	0.23	2.00	0.44	0.64	16.68	22.60	1.36	0.23	71.54	7.89	0.62
NET	5.92	2.58	0.14	0.77	0.15	1.84	0.44	0.64	12.48	17.25		0.17		5.97	

Table B-9. Acid concentration data for 100-g VS/$L_{liq.}$ relaxed batch fermentation.

	C2	C3	C4	IC4	C5	IC5	C6	C7	Total	Aceq	Aceq Ratio	Molar	Avg MW	g C/L	φ
Day	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g aceq/ L_{liq}	g aceq/ g tot acid	mol/L_{liq}	g/mol	g/L_{liq}	mols acid/ mol aceq
0	2.26	1.55	0.08	0.46	0.09	0.15	0.00	0.00	4.59	5.83	1.27	0.07	68.47	2.09	0.69
2	2.92	2.61	0.09	0.47	0.09	0.24	0.06	0.00	6.48	8.33	1.29	0.09	68.96	2.97	0.68
4	4.37	4.06	0.11	0.60	0.10	0.52	0.11	0.04	9.90	12.83	1.30	0.14	69.31	4.56	0.67
6	6.04	4.88	0.13	0.74	0.14	1.12	0.24	0.24	13.53	17.87	1.32	0.19	70.25	6.30	0.65
8	5.95	5.31	0.13	0.77	0.13	0.88	0.16	0.10	13.43	17.49	1.30	0.19	69.58	6.21	0.66
10	6.15	5.79	0.13	0.82	0.12	0.69	0.13	0.08	13.92	17.99	1.29	0.20	69.22	6.40	0.67
12	5.52	5.12	0.14	0.95	0.15	2.02	0.39	0.42	14.71	20.51	1.39	0.20	73.08	7.07	0.59
14	7.44	6.62	0.17	1.18	0.20	2.52	0.49	0.55	19.16	26.53	1.38	0.26	72.70	9.18	0.60
16	6.39	5.34	0.14	0.95	0.16	1.99	0.39	0.46	15.81	21.73	1.37	0.22	72.29	7.54	0.60
18	7.39	5.77	0.15	1.01	0.17	2.10	0.41	0.47	17.47	23.77	1.36	0.24	71.75	8.28	0.62
20	7.35	5.48	0.14	0.96	0.16	1.98	0.39	0.44	16.90	22.86	1.35	0.24	71.45	7.98	0.62
22	7.41	5.25	0.14	0.93	0.16	1.93	0.38	0.42	16.61	22.36	1.35	0.23	71.20	7.82	0.63
24	7.79	5.35	0.17	1.01	0.18	2.06	0.41	0.45	17.43	23.52	1.35	0.24	71.32	8.22	0.62
26	8.10	5.41	0.18	1.05	0.19	2.14	0.42	0.47	17.96	24.22	1.35	0.25	71.30	8.47	0.62
28	8.36	5.41	0.20	1.11	0.20	2.22	0.44	0.49	18.43	24.88	1.35	0.26	71.34	8.70	0.62
NET	6.10	3.86	0.12	0.65	0.12	2.07	0.44	0.49	13.85	19.05		0.19		6.61	

Table B-10. Acid concentration data for 100-g VS/$L_{liq.}$-plus-20-g carboxylate salts/$L_{liq.}$ relaxed batch fermentation. The *target* initial carboxylate salt concentrations were 9 g calcium acetate/$L_{liq.}$, 9 g calcium propionate/$L_{liq.}$, and 2 g butyric acid/$L_{liq.}$.

	C2	**C3**	**C4**	**IC4**	**C5**	**IC5**	**C6**	**C7**	**Total**	**Aceq**	**Aceq Ratio**	**Molar**	**Avg MW**	**g C/L**	**φ**
Day	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g/L_{liq}	g aceq/ L_{liq}	g aceq/ g tot acid	mol/L_{liq}	g/mol	g/L_{liq}	mols acid/ mol aceq
0	7.11	6.72	0.09	2.05	0.11	0.16	0.00	0.00	16.24	20.80	1.28	0.24	68.79	7.43	0.68
2	6.59	6.53	0.08	1.67	0.08	0.13	0.00	0.00	15.07	19.22	1.28	0.22	68.60	6.88	0.69
4	7.17	7.09	0.08	1.82	0.09	0.15	0.00	0.00	16.40	20.92	1.28	0.24	68.62	7.49	0.69
6	7.16	6.64	0.08	1.62	0.09	0.38	0.09	0.00	16.06	20.56	1.28	0.23	68.77	7.35	0.68
8	7.34	6.61	0.08	1.55	0.09	0.49	0.12	0.00	16.29	20.86	1.28	0.24	68.79	7.45	0.68
10	10.03	9.13	0.13	1.93	0.13	1.17	0.31	0.08	22.91	29.80	1.30	0.33	69.50	10.58	0.66
12	9.59	8.65	0.13	1.74	0.13	1.25	0.31	0.11	21.91	28.56	1.30	0.31	69.61	10.13	0.66
14	9.91	8.69	0.13	1.70	0.14	1.30	0.31	0.13	22.32	29.05	1.30	0.32	69.53	10.31	0.66
16	10.97	9.42	0.15	1.85	0.16	1.44	0.34	0.13	24.46	31.79	1.30	0.35	69.47	11.29	0.67
18	10.58	8.84	0.15	1.76	0.16	1.38	0.32	0.13	23.34	30.30	1.30	0.34	69.41	10.76	0.67
20	12.28	10.21	0.18	1.95	0.18	1.58	0.36	0.15	26.89	34.84	1.30	0.39	69.31	12.39	0.67
22	13.18	10.63	0.18	2.02	0.19	1.64	0.37	0.16	28.36	36.61	1.29	0.41	69.14	13.04	0.67
24	12.21	9.71	0.18	1.87	0.18	1.54	0.35	0.16	26.20	33.84	1.29	0.38	69.18	12.05	0.67
26	12.63	9.83	0.20	1.94	0.20	1.62	0.36	0.16	26.94	34.79	1.29	0.39	69.17	12.39	0.67
28	14.17	10.76	0.23	2.20	0.23	1.85	0.41	0.19	30.04	38.82	1.29	0.43	69.19	13.82	0.67
NET	7.05	4.04	0.15	0.15	0.13	1.70	0.41	0.19	13.80	18.02		0.19		6.39	

APPENDIX C

DATA FOR INVESTIGATION OF NUTRIENT FEEDING STRATEGIES IN A COUNTERCURRENT MIXED-ACID MULTI-STAGED FERMENTATION

Table C-1. Transfer data for Train 1 Fermentor 1. Red indicates data was estimated or interpolated based on other data.

Train 1 – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
1.78	6	87.8	35	511.2	473.1	0.960	0.380
3.91	6	109.6	35	500.6	489.9	0.960	0.380
6.05	6	84.8	35	392.1	472.4	0.960	0.380
8.00	6.5	45.4	35	198.6	438.3	0.960	0.380
10.79	6	40.7	35	147.5	430.0	0.960	0.380
12.94	6.5	8.2	35	672.9	417.2	0.960	0.380
15.00	7	30.9	35	151.8	412.2	0.960	0.380
17.79	6.5	2.1	35	470.8	390.9	0.960	0.380
20.03	7	6.2	35	451.7	388.7	0.960	0.380
21.98	7	10.2	35	411.3	365.0	0.960	0.380
24.79	7	3	35	336.8	271.0	0.960	0.380
26.96	0	0	35	198.6	243.4	0.960	0.380
28.35	0	0	35	158.1	255.0	0.960	0.380
31.71	0	0	35	202.8	253.5	0.960	0.380
33.35	0	0	35	107.1	239.8	0.960	0.380
36.19	0	0	35	515.5	293.9	0.960	0.380
38.77	7.5	6.5	35	464.4	327.4	0.960	0.380
40.00				415.5			
40.87	7	14.7	35	259.0	320.1	0.960	0.380
42.00				400.6			
43.13	6.5	39.2	35	51.8	338.2	0.960	0.380
44.00				387.9			
45.00				156.0			
45.74	6.5	68.4	35	73.1	371.6	0.960	0.380
47.00				490.0			
48.02	6.5	88.1	35	126.2	383.2	0.960	0.380
49.00				298.5			

Table C-1. Continued.

Train 1 – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
50.12	7	93.4	35	113.5	394.0	0.960	0.380
51.00				419.8			
52.00				141.1			
52.89	6.5	128.6	35	22.0	446.9	0.960	0.380
54.00				479.3			
55.00				173.0			
56.00				141.1			
57.08	6.5	125.2	35	111.4	429.2	0.960	0.380
58.00				438.9			
59.00				128.4			
59.75	6.5	91.4	35	134.7	408.2	0.960	0.380
61.00				413.4			
61.97	6.5	125.1	35	196.4	452.0	0.960	0.380
64.82	6.5	189.9	35	192.2	510.8	0.960	0.380
66.00				400.6			
66.73	6.5	131.2	35	209.2	428.5	0.960	0.380
68.00				436.8			
68.74	6.5	109.1	35	153.9	426.7	0.960	0.380
71.00				538.9			
71.80	6	133.7	35	134.7	461.2	0.960	0.380
73.00				453.8			
73.79	6	107.5	35	162.4	433.5	0.960	0.380
75.00				487.8			
76.03	6.5	122.5	35	139.0	420.6	0.960	0.380
77.00				443.2			
78.10	6.5	106.6	35	207.1	431.5	0.960	0.380
79.00				513.4			
80.00				196.4			
80.75	6.5	132.8	35	115.6	441.7	0.960	0.380
82.00				432.5			
83.05	6.5	111.9	35	213.4	437.6	0.958	0.345
84.00				259.0			
85.10	6.5	93.7	35	279.4	418.8	0.966	0.420
86.00				485.7			
87.00				204.9			

Table C-1. Continued.

Train 1 – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
87.77	6	98.2	35	70.9	418.0	0.965	0.402
89.00				447.4			
90.07	6.5	128.6	35	198.6	444.9	0.935	0.506
91.00				409.1			
92.10	6.5	124.6	35	181.5	451.4	0.968	0.665
93.00				449.5			
94.00				187.9			
94.75	6.5	124	35	45.4	432.9	0.949	0.831
96.00				358.1			
97.09	6	90.6	35	219.8	412.6	0.958	0.508
98.00				494.2			
99.11	6.5	87.2	35	177.3	414.0	0.964	0.455
100.00				524.0			
101.00				134.7			
101.75	6.5	104.6	35	28.4	417.8	0.925	0.668
103.00				426.1			
104.10	6	113.6	35	259.0	425.9	0.957	0.548
105.00				432.5			
106.10	6	100.8	35	275.1	416.3	0.965	0.448
107.00				366.6			
108.00				236.8			
108.76	6.5	92.2	35	181.5	447.3	0.945	0.408
110.00				551.6			
111.05	6	38.7	35	241.1	371.6	0.959	0.632
112.00				275.1			
113.09	6	147.5	35	190.0	438.2	0.963	0.378
114.00				532.5			
115.00				345.3			
115.76	6	127.6	35	15.6	465.2	0.954	0.369
117.00				485.7			
118.09	6	116.7	35	334.7	427.8	0.961	0.556
119.00				496.3			
120.10	6	123.8	35	179.4	428.8	0.969	0.480
121.00				390.0			
122.00				173.0			

Table C-1. Continued.

Train 1 – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
122.77	6.1	103.8	35	124.1	447.6	0.960	0.847
124.00				453.8			
125.08	6	87.6	35	232.6	425.9	0.963	0.570
126.00				532.5			
127.12	6	136.5	35	185.8	442.4	0.971	0.556
128.00				481.4			
129.00				181.5			
129.74	6.1	141.4	35	90.1	487.0	0.971	0.857
131.00				211.3			
132.07	6	117.5	35	170.9	437.1	0.963	0.602
133.00				500.6			
134.07	6	146.3	35	130.5	457.1	0.965	0.602
135.00				504.8			
136.00				111.4			
136.75	6.5	104.0	35	41.2	436.0	0.966	0.602
138.00				594.2			
139.11	6	149.9	35	262.4	445.0	0.967	0.602
140.00				453.8			
141.06	6	128.5	35	113.5	449.0	0.966	0.812
142.00				496.3			
143.00				173.0			
143.73	6.5	141.4	35	62.4	460.1	0.970	0.616
145.00				404.9			
146.04	6.5	88.2	35	149.6	412.9	0.952	0.331
147.00				455.9			
148.06	6.5	119.0	35	177.3	444.0	0.965	0.329
149.00				421.9			
150.00				181.5			
150.74	6.5	108.4	35	143.3	428.2	0.964	0.579
152.00				490.0			
153.17	6	99.0	35	232.6	452.8	0.967	0.603
154.00				562.3			
155.07	6	86.5	35	230.5	410.4	0.965	0.490
156.00				613.3			
157.00				105.0			
157.74	6.5	93.6	35	81.6	408.6	0.963	0.590

Table C-1. Continued.

Train 1 – Fermentor 1							
Day	**pH**	**Liquid**	**Feedstock**	**Gas**	**Total**	***M* (liq.**	***I* (liq.**
		g	g	cm^3	g	0	0
159.00				5.0			
160.08	6	118.4	35	326.2	443.8	0.964	0.537
161.00				521.9			
162.08	6	107.7	35	202.8	444.5	0.964	0.799

Table C-2. Transfer data for Train 1 Fermentor 2. Red indicates data was estimated or interpolated based on other data.

Train 1 – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
1.78	5.5	195	149.2	105.0	480.5
3.91	6	178.5	144.2	151.8	494.0
6.05	6	163.2	146.6	136.9	498.2
8.00	6.5	141.5	151.9	147.5	493.3
10.79	6	121.1	148.3	119.9	441.9
12.94	6	118.3	168	762.2	465.5
15.00	6.5	99.3	140.3	100.7	424.6
17.79	6.5	96.7	147.8	249.6	437.3
20.03	7	76.5	141.6	190.0	434.9
21.98	6.5	77.2	213.8	194.3	426.1
24.79	6.5	46.2	127	226.2	406.6
26.96	6.5	58.3	102.4	270.9	424.7
28.35	6.5	60.1	114	214.0	418.2
31.71	6.5	43.8	112.5	411.3	422.7
33.35	6.5	100.9	98.8	5.0	488.7
36.19	6	100.1	123.9	504.8	493.5
38.77	6.5	124.8	179.9	287.9	487.2
40.00				198.557	
40.87	6	144.8	164.4	214.0	513.8
42.00				32.651	
43.13	6	177	158	409.1	529.3
44.00				226.2	
45.00				232.6	
45.74	6	192.6	162.2	143.3	538.1
47.00				239.0	
48.02	6.5	196.8	154.1	194.3	546.4
49.00				190.0	
50.12	6.5	250.7	159.6	185.8	600.2
51.00				309.2	
52.00				247.5	
52.89	6	237.7	177.3	147.5	613.0
54.00				270.9	
55.00				217.7	

Table C-2. Continued.

Train 1 – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
56.00				215.6	
57.08	6	216.7	163	241.1	546.2
58.00				262.4	
59.00				51.8	
59.75	6.5	263.9	175.8	143.3	615.8
61.00				249.6	
61.97	6	319.2	185.9	134.7	654.9
64.82	6.5	325.3	179.9	200.7	678.3
66.00				185.8	
66.73	6.5	234.3	156.3	141.1	566.9
68.00				217.7	
68.74	6.5	264	176.6	126.2	594.3
71.00				436.8	
71.80	6	241.3	186.5	207.1	592.3
73.00				245.4	
73.79	6	228.2	185.6	149.6	570.2
75.00				213.4	
76.03	6.5	239.4	157.1	179.4	584.6
77.00				194.3	
78.10	6	247	183.9	260.2	580.9
79.00				277.3	
80.00				253.9	
80.75	6	247.7	167.9	194.3	584.4
82.00				239.0	
83.05	6.5	225.1	184.7	262.4	571.5
84.00				214.0	
85.10	6	225.3	184.1	277.3	568.7
86.00				232.6	
87.00				222.0	
87.77	6	252.4	178.8	141.1	582.1
89.00				187.9	
90.07	6.5	257.1	175.3	226.2	576.2
91.00				185.8	
92.10	6	243.9	185.8	209.2	580.0
93.00				158.1	
94.00				192.2	

Table C-2. Continued.

Train 1 – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
94.75	6	226.6	167.9	124.1	562.0
96.00				209.2	
97.09	6	220.6	181	349.6	561.5
98.00				162.4	
99.11	6	222.6	185.8	183.7	555.9
100.00				194.3	
101.00				145.4	
101.75	6	236.8	172.2	98.6	551.6
103.00				341.1	
104.10	6	220	171.3	214.0	572.1
105.00				187.9	
106.10	6	258.9	174.5	311.3	609.1
107.00				285.8	
108.00				300.7	
108.76	6.1	181.6	214.1	151.8	528.4
110.00				385.7	
111.05	6	249.7	191.9	222.0	587.2
112.00				194.3	
113.09	6	282.2	149.7	200.7	615.3
114.00				260.2	
115.00				230.5	
115.76	6.1	236.2	196.6	170.9	577.4
117.00				304.9	
118.09	6	233.8	170.1	209.2	580.5
119.00				264.5	
120.10	6	256.1	164	239.0	588.9
121.00				211.3	
122.00				145.4	
122.77	6.1	238.8	202.8	102.8	598.5
124.00				245.4	
125.08	6	250.4	197.3	185.8	590.2
126.00				287.9	
127.12	6	298.1	164.9	5.0	626.7
128.00				260.2	
129.00				162.4	
129.74	6.1	265.9	204.6	98.6	607.4

Table C-2. Continued.

Train 1 – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
131.00				190.0	
132.07	6	263.2	178.6	128.4	607.3
133.00				262.4	
134.07	6	243.2	169.8	168.8	500.9
135.00				215.6	
136.00				156.0	
136.75	6.5	258.4	191	64.6	599.8
138.00				319.8	
139.11	6	258.3	154.1	249.6	591.6
140.00				185.8	
141.06	6	267.6	179.5	128.4	603.9
142.00				266.6	
143.00				194.3	
143.73	6	227.5	177.7	100.7	567.8
145.00				173.0	
146.04	6.5	248.9	183.7	187.9	593.7
147.00				217.7	
148.06	6.5	235.6	184	185.8	571.4
149.00				198.6	
150.00				122.0	
150.74	6.5	263.1	178.8	175.2	602.5
152.00				241.1	
153.17	6	217.6	212.8	292.1	577.7
154.00				266.6	
155.07	6	216	182.9	385.7	559.3
156.00				326.2	
157.00				239.0	
157.74	6	251.7	174	273.0	590.7
159.00				5.0	
160.08	6	272.7	184.4	315.5	618.0
161.00				300.7	
162.08	6	245.9	195.8	273.0	610.1

Table C-3. Transfer data for Train 1 Fermentor 3. Red indicates data was estimated or interpolated based on other data.

Train 1 – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
1.78	5	198.2	134.7	70.9	490.3
3.91	6	203.5	159.7	79.4	470.9
6.05	6	188.9	181	109.2	495.2
8.00	6	145.9	203.7	13.5	463.4
10.79	6	172.4	169.1	90.1	483.7
12.94	6	130.7	215.2	768.6	454.8
15.00	6	143.2	165.6	73.1	462.3
17.79	6	138.6	188.6	102.8	473.5
20.03	6.5	127.4	200	109.2	448.6
21.98	6.5	109.4	262.7	134.7	452.3
24.79	6	4:48	187.4	266.6	487.3
26.96	6	130.6	168.8	270.9	465.0
28.35	6.5	122.9	172.1	132.6	455.2
31.71	6	193.8	191.4	283.6	541.4
33.35	6	202.7	186.6	336.8	558.5
36.19	6	189.5	217.3	364.5	553.7
38.77	6	221.2	242.3	292.1	574.3
40.00				264.494	
40.87	6	233.1	233.4	233.0	603.9
42.00				249.605	
43.13	6	243.6	210.3	260.2	599.4
44.00				292.1	
45.00				264.5	
45.74	6	252.3	207.7	177.3	591.3
47.00				304.9	
48.02	6.5	309.6	203.7	173.0	634.8
49.00				207.1	
50.12	6	321.2	209.1	222.0	646.6
51.00				217.7	
52.00				283.6	
52.89	6	256	252.6	224.1	602.6
54.00				251.7	
55.00				290.0	

Table C-3. Continued.

Train 1 – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
56.00				281.5	
57.08	6	320.5	192.5	343.2	564.7
58.00				209.2	
59.00				49.7	
59.75	6	364.5	227.7	173.0	677.5
61.00				185.8	
61.97	6	292.8	221.6	151.8	614.6
64.82	6.5	271.2	232.9	230.5	604.7
66.00				156.0	
66.73	6	294.8	188.9	124.1	616.3
68.00				179.4	
68.74	6	293.9	206.9	260.2	615.0
71.00				224.1	
71.80	6	274.4	237.5	232.6	591.8
73.00				392.1	
73.79	6	288.9	227.6	194.3	634.8
75.00				211.3	
76.03	6	288.4	202.3	330.4	606.6
77.00				168.8	
78.10	6	292	217.8	326.2	598.3
79.00				392.1	
80.00				234.7	
80.75	6	275.6	204.6	141.1	604.5
82.00				222.0	
83.05	6.5	275.3	231.1	219.8	589.5
84.00				233.0	
85.10	6	288.4	227.5	300.7	628.9
86.00				162.4	
87.00				270.9	
87.77	6	284.6	208.5	153.9	608.3
89.00				202.8	
90.07	6	284.1	194.4	338.9	602.9
91.00				160.3	
92.10	6	270	221.9	296.4	593.4
93.00				124.1	
94.00				292.1	

Table C-3. Continued.

Train 1 – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
94.75	6	275.9	203.3	177.3	600.8
96.00				177.3	
97.09	6	264.3	221.9	402.7	601.5
98.00				149.6	
99.11	6	258.1	219.1	277.3	587.9
100.00				179.4	
101.00				275.1	
101.75	6	282.6	189	266.6	605.8
103.00				304.9	
104.10	6	312.6	223.4	233.0	628.1
105.00				226.2	
106.10	6	235.4	224.7	334.7	560.5
107.00				245.4	
108.00				258.1	
108.76	6.1	295.3	260.9	126.2	616.2
110.00				239.0	
111.05	6	321.2	229.4	298.5	645.4
112.00				177.3	
113.09	6	285.4	182.8	281.5	616.1
114.00				177.3	
115.00				287.9	
115.76	6.1	289.1	237.8	166.7	586.5
117.00				239.0	
118.09	6	293.7	216.8	247.5	625.5
119.00				302.8	
120.10	6	306.7	196.8	245.4	603.7
121.00				230.5	
122.00				192.2	
122.77	6.1	301.6	262.5	109.2	613.7
124.00				243.2	
125.08	6	334.4	237.1	245.4	658.0
126.00				213.4	
127.12	6	309.4	193.5	253.9	626.0
128.00				190.0	
129.00				204.9	
129.74	6.1	316	246.1	124.1	603.9

Table C-3. Continued.

Train 1 – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
131.00				179.4	
132.07	6	306.5	222.7	211.3	633.8
133.00				198.6	
134.07	6	306.2	227.5	330.4	611.9
135.00				200.7	
136.00				287.9	
136.75	6	308.4	232.4	143.3	615.1
138.00				268.7	
139.11	6	307.7	187.4	302.8	610.3
140.00				170.9	
141.06	6	272.2	215.8	213.4	580.7
142.00				311.3	
143.00				326.2	
143.73	6	299.9	218	147.5	592.1
145.00				173.0	
146.04	6	275.3	228.5	300.7	592.0
147.00				209.2	
148.06	6	303.7	219.8	283.6	608.3
149.00				185.8	
150.00				256.0	
150.74	6	291.6	218.2	94.3	620.2
152.00				307.0	
153.17	6	263.7	272.9	270.9	595.0
154.00				451.7	
155.07	6	295.8	226.2	304.9	639.9
156.00				481.4	
157.00				253.9	
157.74	6	323.7	213	153.9	629.4
159.00				5.0	
160.08	6	326.9	229.7	249.6	611.6
161.00				351.7	
162.08	6	292.3	260	209.2	618.9

Table C-4. Transfer data for Train 1 Fermentor 4. Red indicates data was estimated or interpolated based on other data.

Train 1 – Fermentor 4									
Day	**pH**	**Liquid removed**	**Cake added**	**Cake removed**	**Liq. added**	**Gas**	**Total ferm.**	***M* (cake removed)**	***I* (cake removed)**
		g	g	g	mL	cm^3	g	0	0
0.00	5.5	197.0	27.9	0.0	175		449.6	0.830	
1.78	5.5	179.4	126.8	99.3	175	115.6	451.9	0.830	
3.91	5.5	201.4	127.1	97.0	175	88.0	471.3	0.830	
6.05	6	178.0	187.3	181.3	175	149.6	472.0	0.830	
8.00	6.5	188.0	221.2	200.1	175	92.2	466.9	0.830	
10.79	6	184.9	180.4	168.4	175	60.3	472.9	0.830	
12.94	6	168.5	239.3	244.5	175	90.1	473.7	0.830	
15.00	6	179.7	184.7	194.2	175	60.3	489.2	0.830	
17.79	6	156.7	226.8	268.8	175	160.3	471.7	0.830	
20.03	6	155.5	221.2	241.7	175	90.1	476.0	0.830	
21.98	6	136.5	252.6	287.5	175	413.4	471.4	0.830	
24.79	6	167.1	246.6	281.2	175	662.2	501.7	0.830	
26.96	6	159.3	203.2	241.1	300	181.5	497.2	0.830	
28.35	6.5	250.7	204.4	239.5	300	266.6	585.8	0.830	
31.71	5.5	261.8	239.0	249.3	300	1428.0	589.8	0.830	
33.35	6	262.7	242.4	262.1	300	819.6	613.5	0.830	
36.19	6	271.3	281.5	334.7	300	558.0	624.5	0.830	
38.77	6	284.0	266.7	266.7	300	792.0	653.6	0.830	
40.00						215.57		0.830	
40.87	6	286.2	284.1	276.6	300	175.0	641.5	0.830	
42.00						262.37		0.830	
43.13	6	298.5	266.1	324.0	300	813.3	656.4	0.830	
44.00						385.7		0.830	
45.00						764.3		0.830	
45.74	5.5	339.5	246.7	223.9	300	498.5	643.6	0.830	
47.00						466.6		0.830	
48.02	6	356.5	228.9	177.0	300	655.9	620.3	0.830	
49.00						187.9		0.830	
50.12	6	307.1	231.5	233.1	300	589.9	605.7	0.830	
51.00						428.3		0.830	
52.00						455.9		0.830	
52.89	6	285.0	257.4	223.4	300	198.6	590.0	0.842	0.301

Table C-4. Continued.

Train 1 – Fermentor 4									
Day	**pH**	**Liquid removed**	**Cake added**	**Cake removed**	**Liquid added**	**Gas**	**Total ferm.**	***M* (cake removed)**	***I* (cake removed)**
		g	g	g	mL	cm^3	g	0	0
54.00						521.9			
55.00						249.6			
56.00						170.9			
57.08	6	384.7	186.7	130.3	300	175.2	628.3	0.806	0.263
58.00						226.2			
59.00						124.1			
59.75	6	304.5	220.9	209.1	300	198.6	592.7	0.812	0.283
61.00						364.5			
61.97	6	301.8	232	203.0	300	222.0	590.6	0.827	0.290
64.82	6.5	299.4	245.1	222.0	300	245.4	606.7	0.823	0.292
66.00						196.4			
66.73	6	328.6	210.4	205.6	300	160.3	623.8	0.834	0.251
68.00						270.9			
68.74	6	299.4	228	216.7	300	145.4	594.4	0.842	0.268
71.00						672.9			
71.80	6	316.1	229.6	210.3	300	126.2	605.2	0.842	0.276
73.00						224.1			
73.79	6	305.2	257.5	220.4	300	98.6	603.5	0.843	0.279
75.00						175.2			
76.03	6	306.3	220.5	236.6	300	128.4	628.8	0.837	0.260
77.00						119.9			
78.10	6	312.6	219.1	204.4	300	141.1	597.9	0.846	0.261
79.00						204.9			
80.00						170.9			
80.75	6	298.4	233.5	212.2	300	113.5	592.8	0.847	0.272
82.00						115.6			
83.05	6	316.3	220.8	209.7	300	168.8	608.4	0.827	0.280
84.00						175.0			
85.10	6	295.8	250	213.8	300	175.2	594.0	0.839	0.243
86.00						100.7			
87.00						145.4			
87.77	6	310.3	232.2	238.5	300	92.2	626.6	0.832	0.221
89.00						100.7			
90.07	6	301.9	213.2	215	300	147.5	603.7	0.828	0.383
91.00						124.1			
92.10	6	299	234.4	218.8	300	115.6	594.9	0.851	0.284

Table C-4. Continued.

Train 1 – Fermentor 4									
Day	**pH**	**Liquid removed**	**Cake added**	**Cake removed**	**Liquid added**	**Gas**	**Total ferm.**	***M* (cake removed)**	***I* (cake removed)**
		g	g	g	mL	cm^3	g	0	0
93.00						75.2			
94.00						139.0			
94.75	6	314.2	228.2	219.4	300	79.4	612.6	0.846	0.353
96.00						132.6			
97.09	6	286.3	249.4	232.9	300	181.5	593.8	0.832	0.350
98.00						51.8			
99.11	6	309.6	239.2	230.2	300	132.6	622.4	0.845	0.325
100.00						119.9			
101.00						134.7			
101.75	6	312	212.2	214.7	300	83.7	614.5	0.827	0.352
103.00						347.4			
104.10	6	260.4	228.4	234.2	300	175.0	572.4	0.836	0.362
105.00						102.8			
106.10	6	314.8	238.3	206.7	300	149.6	600.9	0.824	0.371
107.00						164.5			
108.00						173.0			
108.76	6.1	304.3	233.4	226.6	300	88.0	608.6	0.848	0.336
110.00						166.7			
111.05	6	305.1	234.2	219.7	300	128.4	606.1	0.845	0.309
112.00						136.9			
113.09	6	296.1	213.5	225.8	300	230.5	608.4	0.811	0.350
114.00						209.2			
115.00						239.0			
115.76	6.1	312.7	210.3	190.1	300	141.1	592.5	0.854	0.330
117.00						156.0			
118.09	6	307.5	244.1	209.4	300	168.8	595.3	0.848	0.306
119.00						207.1			
120.10	6	324.4	193.8	187.9	300	168.8	618.5	0.835	0.311
121.00						160.3			
122.00						153.9			
122.77	6.1	315.2	222.9	197	300	88.0	590.6	0.832	0.351
124.00						126.2			
125.08	6	315.1	236.5	198.9	300	147.5	592.3	0.832	0.290
126.00						277.3			
127.12	6	314.7	210.1	203.3	300	213.4	607.9	0.820	0.287

Table C-4. Continued.

Train 1 – Fermentor 4									
Day	**pH**	**Liquid removed**	**Cake added**	**Cake removed**	**Liquid added**	**Gas**	**Total ferm.**	***M* (cake removed)**	***I* (cake removed)**
		g	g	g	mL	cm^3	g	0	0
128.00						187.9			
129.00						160.3			
129.74	6.1	320.3	200.3	172.6	300	83.7	592.6	0.823	0.415
131.00						151.8			
132.07	6	308.9	240.9	206.8	300	124.1	593.2	0.835	0.327
133.00						264.5			
134.07	6	316.3	218.5	212.6	300	213.4	610.4	0.802	0.327
135.00						268.7			
136.00						168.8			
136.75	6	289	219.4	204.9	300	79.4	574.5	0.841	0.249
138.00						232.6			
139.11	6	291.8	190	186.5	300	132.6	588.3	0.840	0.317
140.00						211.3			
141.06	6	296.6	222	219.2	300	164.5	593.8	0.823	0.323
142.00						315.5			
143.00						200.7			
143.73	6	295.5	203.8	204.2	300	96.5	595.9	0.857	0.236
145.00						187.9			
146.04	6	300.5	227.5	208.9	300	134.7	593.2	0.829	0.445
147.00						149.6			
148.06	6	319.2	215.9	202.1	300	88.0	605.4	0.833	0.418
149.00						115.6			
150.00						122.0			
150.74	6	300	240.8	214.5	300	98.6	594.2	0.840	0.354
152.00						153.9			
153.17	6	288.8	243.4	248.7	300	181.5	616.3	0.827	0.535
154.00						245.4			
155.07	6	323.7	257.1	214	300	156.0	616.3	0.854	0.349
156.00						294.3			
157.00						136.9			
157.74	6	319.1	218.7	230.5	300	90.1	630.9	0.824	0.469
159.00						5.0			
160.08	6	333	196	160.3	300	166.7	597.3	0.830	0.317
161.00						202.8			
162.08	6	289.3	243.3	202.2	300	124.1	565.7	0.826	0.384

Table C-5. Transfer data for Train 2 Fermentor 1. Red indicates data was estimated or interpolated based on other data.

Train 2 – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
0.00	6.5	88.3	35		449.9	0.960	0.388
1.78	6	67.5	35	151.8	462.9	0.960	0.388
3.89	6	93.1	35	183.7	496.0	0.960	0.388
6.00	6.5	76.8	35	163.0	471.2	0.960	0.388
8.02	6	101.9	35	47.5	498.9	0.960	0.388
10.80	6	20.9	35	211.3	440.7	0.960	0.388
12.98	6	7.3	35	168.8	414.5	0.960	0.388
14.99	7	21.2	35	260.2	426.1	0.960	0.388
17.79	6.5	2.6	35	228.3	394.4	0.960	0.388
20.01	6.5	0	35	298.5	384.0	0.960	0.388
22.00	6.5	10.1	35	249.6	383.2	0.960	0.388
24.78	6.5	3.5	35	268.7	292.8	0.960	0.388
26.95	7	2.4	35	243.2	266.8	0.960	0.388
29.02	6.5	18.5	35	304.9	333.5	0.960	0.388
31.70	7	10.1	35	341.1	271.2	0.960	0.388
33.32	0	0	35	217.7	227.0	0.960	0.388
36.19	6.5	5.2	35	330.4	339.3	0.960	0.388
38.77	6.5	1.3	35	338.9	317.7	0.960	0.388
40.00				241.1			
40.87	6.5	39.9	35	163.0	348.4	0.960	0.388
42.00				170.9			
43.12	7	55	35	115.6	377.7	0.960	0.388
44.00				143.3			
45.00				77.3			
45.74	6.5	80.8	35	26.3	383.6	0.960	0.388
47.00				153.9			
48.01	6.5	34.5	35	124.1	372.2	0.960	0.388
49.00				177.3			
50.12	7	56.3	35	68.8	392.5	0.960	0.388
51.00				202.8			
52.00				134.7			
52.89	6.5	80.1	35	13.5	405.9	0.960	0.388
54.00				262.4			
55.00				130.5			
56.00				32.7			

Table C-5. Continued.

Train 2 – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
57.05	6	127.1	35	30.5	444.4	0.960	0.388
58.00				262.4			
59.00				56.0			
59.75	6.5	117.9	35	83.7	441.6	0.960	0.388
61.00				234.7			
61.96	6.5	213	35	81.6	521.2	0.960	0.388
64.82	6.5	223.5	35	151.8	560.6	0.960	0.388
66.00				236.8			
66.72	6	99.1	35	85.8	423.6	0.960	0.388
68.00				256.0			
68.73	6.5	137.2	35	98.6	465.4	0.960	0.388
71.00				619.7			
71.78	6.5	166.7	35	122.0	494.5	0.960	0.388
73.00				251.7			
73.78	6	174.2	35	94.3	519.9	0.960	0.388
75.00				281.5			
76.03	6.5	180	35	136.9	481.3	0.960	0.388
77.00				207.1			
78.08	6	157.8	35	166.7	479.0	0.960	0.388
79.00				226.2			
80.00				126.2			
80.74	6.5	124	35	75.2	450.6	0.960	0.388
82.00				264.5			
83.03	6.5	206.4	35	92.2	514.3	0.966	0.354
84.00				163.0			
85.08	6	130	35	145.4	462.4	0.963	0.430
86.00				260.2			
87.00				156.0			
87.76	6.5	147	35	66.7	486.0	0.967	0.380
89.00				319.8			
90.06	6.5	141.8	35	145.4	502.6	0.948	0.412
91.00				338.9			
92.08	6.5	133.8	35	113.5	466.2	0.952	0.814
93.00				226.2			
94.00				113.5			

Table C-5. Continued.

Train 2 – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
94.73	6.5	140.5	35	47.5	464.0	0.967	0.500
96.00				164.5			
97.09	6.5	118.3	35	232.6	476.2	0.960	0.750
98.00				234.7			
99.10	6	132.8	35	158.1	491.8	0.963	0.500
100.00				349.6			
101.00				141.1			
101.73	6.5	145.3	35	64.6	476.5	0.925	0.668
103.00				283.6			
104.08	6	179.9	35	163.0	538.1	0.948	0.607
105.00				268.7			
106.10	6	133.1	35	204.9	492.8	0.953	0.574
107.00				309.2			
108.00				160.3			
108.75	6.1	140.2	35	79.4	483.7	0.959	0.104
110.00				279.4			
111.06	6	140.7	35	107.1	517.5	0.966	0.607
112.00				75.2			
113.08	6	175.3	35	198.6	529.6	0.965	0.423
114.00				262.4			
115.00				149.6			
115.76	6.1	175.2	35	73.1	530.8	0.965	0.330
117.00				290.0			
118.08	6	163.3	35	151.8	500.6	0.966	0.745
119.00				268.7			
120.11	6	144.2	35	141.1	494.9	0.966	0.915
121.00				277.3			
122.00				105.0			
122.76	6.1	131.6	35	56.0	479.3	0.971	0.592
124.00				281.5			
125.09	6.1	182.0	35	130.5	524.2	0.966	0.497
126.00				290.0			
127.10	6	185.7	35	98.6	501.2	0.960	0.563
128.00				270.9			
129.00				107.1			

Table C-5. Continued.

Train 2 – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
129.73	6	229.0	35	45.4	524.8	0.970	0.584
131.00				366.6			
132.06	6	160.7	35	164.5	508.1	0.959	0.583
133.00				330.4			
134.05	6	167.5	35	175.2	506.6	0.963	0.392
135.00				290.0			
136.00				162.4			
136.73	6.5	177.2	35	81.6	504.2	0.970	0.440
138.00				256.0			
139.10	6	165.7	35	224.1	516.6	0.964	0.915
140.00				319.8			
141.03	6	185.8	35	160.3	499.4	0.964	0.786
142.00				232.6			
143.00				128.4			
143.76	6	144.9	35	62.4	497.0	0.968	0.688
145.00				370.8			
146.03	6	166.9	35	179.4	528.0	0.967	0.646
147.00				362.3			
148.04	6.5	144.6	35	196.4	475.9	0.961	0.790
149.00				362.3			
150.00				160.3			
150.75	6	136.2	35	124.1	496.6	0.963	0.621
152.00				328.3			
153.02	6	208.0	35	215.6	578.5	0.960	0.594
154.00				377.2			
155.04	6	185.4	35	183.7	524.0	0.963	0.588
156.00				368.7			
157.00				168.8			
157.72	6	185.8	35	105.0	537.7	0.965	0.715
159.00				5.0			
160.07	6	243.7	35	326.2	611.2	0.964	0.604
161.00				396.4			
162.06	6	197.4	35	207.1	551.4	0.965	0.874

Table C-6. Transfer data for Train 2 Fermentor 2. Red indicates data was estimated or interpolated based on other data.

Train 2 – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
0.00	6	168	96.6		449.7
1.78	6	198.8	130.4	122.0	479.6
3.89	6	204.2	137.9	170.9	505.0
6.00	6.5	150.3	129.4	209.2	488.2
8.02	6.5	177.5	132	128.4	512.2
10.80	6.5	118.8	154.8	204.9	464.2
12.98	6	131	142.2	56.0	468.8
14.99	7	102.7	139.9	190.0	448.8
17.79	6.5	93.8	126.8	279.4	433.8
20.01	6.5	86.3	119	477.2	445.2
22.00	6.5	95.5	208.1	341.1	444.4
24.78	6.5	71.1	124.3	234.7	434.3
26.95	7	141.8	99.4	290.0	500.5
29.02	6.5	78.5	150	214.0	426.8
31.70	6.5	33.1	96.1	660.1	423.7
33.32	6.5	140.4	62	592.1	527.2
36.19	6	119.5	169.1	492.1	488.1
38.77	6.5	151.4	151.4	392.1	507.3
40.00				207.1	
40.87	6.5	182	143.5	214.0	545.9
42.00				207.1	
43.12	6.5	185.7	157.7	230.5	545.9
44.00				196.4	
45.00				247.5	
45.74	6	175.7	137.8	190.0	541.9
47.00				241.1	
48.01	6.5	201.1	172.7	202.8	559.9
49.00				183.7	
50.12	6.5	208.3	171.2	190.0	562.6
51.00				209.2	
52.00				187.9	
52.89	6.5	252.2	160.8	164.5	626.8
54.00				277.3	
55.00				321.9	
56.00				287.9	

Table C-6. Continued.

Train 2 – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
57.05	6	246.2	152.3	309.2	587.1
58.00				253.9	
59.00				36.9	
59.75	6	330.2	158.7	128.4	694.0
61.00				281.5	
61.96	6.5	364.5	143.2	170.9	732.5
64.82	6.5	224.8	172.1	315.5	586.5
66.00				275.1	
66.72	6	272.9	159.5	39.0	622.4
68.00				366.6	
68.73	6	297.5	163.2	228.3	647.9
71.00				209.2	
71.78	6	321.8	162.8	281.5	670.1
73.00				364.5	
73.78	6	289.4	180.7	175.2	639.8
75.00				270.9	
76.03	6.5	286.4	136.3	224.1	628.8
77.00				204.9	
78.08	6	262.7	156.2	356.0	589.0
79.00				285.8	
80.00				362.3	
80.74	6	326	161.6	222.0	667.1
82.00				356.0	
83.03	6	256.9	142.9	387.9	600.5
84.00				214.0	
85.08	6	292	167.4	326.2	543.2
86.00				315.5	
87.00				304.9	
87.76	6	312.6	174	253.9	658.8
89.00				362.3	
90.06	6	273.7	195.8	426.1	637.4
91.00				353.8	
92.08	6	268.3	167.4	451.7	643.2
93.00				287.9	
94.00				373.0	
94.73	6	331	158.5	268.7	684.2

Table C-6. Continued.

Train 2 – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
96.00				330.4	
97.09	6	295.9	192.9	513.4	700.9
98.00				234.7	
99.10	6	281.8	194	219.8	620.9
100.00				394.2	
101.00				292.1	
101.73	6.5	348	166.2	345.3	683.5
103.00				377.2	
104.08	6	298.3	193.2	214.0	671.8
105.00				343.2	
106.10	6	289.7	194.7	447.4	647.9
107.00				349.6	
108.00				381.5	
108.75	6.1	322.5	178.5	317.7	672.7
110.00				375.1	
111.06	6	334.7	211.8	398.5	674.5
112.00				200.7	
113.08	6	339.3	189.3	341.1	680.7
114.00				234.7	
115.00				147.5	
115.76	6.1	307.7	190.6	181.5	640.1
117.00				236.8	
118.08	6	302	172.3	283.6	658.1
119.00				290.0	
120.11	6	283.8	185.7	202.8	630.2
121.00				332.6	
122.00				417.6	
122.76	6.1	332.8	182.7	317.7	675.2
124.00				279.4	
125.09	6.1	311.2	177.2	324.1	656.8
126.00				247.5	
127.10	6	321.1	150.5	147.5	647.9
128.00				247.5	
129.00				190.0	
129.73	6	317.6	130.8	164.5	663.7
131.00				430.4	

Table C-6. Continued.

Train 2 – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
132.06	6	313.9	182.4	341.1	666.4
133.00				277.3	
134.05	6	312.2	174.1	222.0	649.5
135.00				213.4	
136.00				143.3	
136.73	6.5	331.8	162	96.5	665.3
138.00				249.6	
139.10	6	307.2	185.9	241.1	634.8
140.00				211.3	
141.03	6	302.5	148.6	194.3	633.2
142.00				236.8	
143.00				290.0	
143.76	6	337.9	187.1	202.8	686.3
145.00				364.5	
146.03	6	283.1	196.1	411.3	607.5
147.00				358.1	
148.04	6	304	166.3	215.6	638.0
149.00				296.4	
150.00				239.0	
150.75	6	384.6	195.4	253.9	724.6
152.00				300.7	
153.02	6	329.9	205.5	298.5	660.7
154.00				390.0	
155.04	6	342.7	173.6	232.6	665.6
156.00				351.7	
157.00				296.4	
157.72	6	420.8	186.9	273.0	735.4
159.00				5.0	
160.07	6	377.8	202.5	526.1	698.2
161.00				273.0	
162.06	6	305.8	189	168.8	634.1

Table C-7. Transfer data for Train 2 Fermentor 3. Red indicates data was estimated or interpolated based on other data.

Train 2 – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
0.00	5.5	192.9	107.2		450.2
1.78	5.5	207.8	140.1	88.0	477.7
3.89	5.5	222	167.6	85.8	495.2
6.00	6	172.3	191.3	94.3	478.7
8.02	6.5	193.9	190.7	117.7	481.3
10.80	6	173	224.2	555.9	469.1
12.98	6	154.2	204	85.8	478.8
14.99	6.5	141	210	88.0	452.5
17.79	6	150.9	190.8	202.8	459.3
20.01	6	147.3	201.9	360.2	459.3
22.00	6	129.7	271.6	275.1	451.1
24.78	6	204.6	211.5	321.9	535.1
26.95	6.5	134.6	182.1	196.4	463.9
29.02	6	134.7	222.3	209.2	445.8
31.70	6	238.4	210.7	345.3	548.7
33.32	6.5	195.4	172.8	479.3	549.4
36.19	6	214.6	245.7	298.5	558.3
38.77	6	251.9	231.3	398.5	616.1
40.00				196.4	
40.87	6	251.9	231.4	326.0	590.6
42.00				113.5	
43.12	6.5	246.9	241.9	268.7	584.4
44.00				287.9	
45.00				351.7	
45.74	6	266.5	228	196.4	607.6
47.00				345.3	
48.01	6	273.3	255.5	243.2	607.6
49.00				215.6	
50.12	6.5	332.2	249.5	222.0	661.5
51.00				236.8	
52.00				287.9	
52.89	6	291.8	259.4	294.3	650.0
54.00				415.5	
55.00				326.2	

Table C-7. Continued.

Train 2 – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
56.00				247.5	
57.05	5.5	401.4	217.2	253.9	644.3
58.00				183.7	
59.00				34.8	
59.75	6	443.6	246.5	124.1	734.1
61.00				228.3	
61.96	6	294.7	235.2	307.0	612.7
64.82	6	324.2	257.8	390.0	644.2
66.00				298.5	
66.72	5.5	358.1	233	239.0	663.7
68.00				236.8	
68.73	6	378.7	237.6	302.8	655.1
71.00				751.6	
71.78	6	342.6	235.1	317.7	614.7
73.00				377.2	
73.78	6	340.5	255.1	219.8	635.6
75.00				194.3	
76.03	6	298.3	202.7	251.7	614.8
77.00				156.0	
78.08	5.5	374	206.5	366.6	621.2
79.00				330.4	
80.00				341.1	
80.74	6	319.2	226.7	294.3	610.8
82.00				385.7	
83.03	6	355	210.5	398.5	596.0
84.00				326.0	
85.08	6	366	242.6	368.7	633.6
86.00				249.6	
87.00				421.9	
87.76	6	348.4	244.2	285.8	625.5
89.00				419.8	
90.06	6	354.9	283.5	509.1	642.2
91.00				402.7	
92.08	6	389.1	266.3	477.2	661.5
93.00				387.9	
94.00				366.6	
94.73	6	418.9	235.7	183.7	690.6

Table C-7. Continued.

Train 2 – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
96.00				115.6	
97.09	6	325.6	321.9	421.9	611.5
98.00				319.8	
99.10	6	390.1	257.1	315.5	688.5
100.00				468.7	
101.00				443.2	
101.73	6	382.5	225.7	347.4	651.6
103.00				428.3	
104.08	6	357.5	290.7	326.0	618.4
105.00				364.5	
106.10	6	380.5	276.9	447.4	651.4
107.00				428.3	
108.00				421.9	
108.75	6.1	388.6	252.7	222.0	667.7
110.00				328.0	
111.06	6	374.8	261.3	287.9	668.2
112.00				375.1	
113.08	6	356.5	254.5	507.0	629.4
114.00				226.2	
115.00				375.1	
115.76	6.1	359.9	247	215.6	649.3
117.00				409.1	
118.08	6	338.2	252.4	449.5	627.3
119.00				239.0	
120.11	6	381.7	256.1	421.9	650.2
121.00				377.2	
122.00				404.9	
122.76	6.1	366.6	249.1	196.4	661.2
124.00				428.3	
125.09	6.1	363.2	246.8	273.0	649.8
126.00				290.0	
127.10	6	371.4	201.3	343.2	627.3
128.00				215.6	
129.00				330.4	
129.73	6	379.4	200.9	168.8	646.5
131.00				407.0	

Table C-7. Continued.

Train 2 – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
132.06	6	361	258.9	273.0	623.8
133.00				234.7	
134.05	6	371.3	235.4	185.8	659.8
135.00				207.1	
136.00				319.8	
136.73	6	349.4	219.5	207.1	637.1
138.00				162.4	
139.10	6	339.5	237.5	215.6	612.8
140.00				190.0	
141.03	6	394.2	203.3	200.7	645.1
142.00				885.6	
143.00				296.4	
143.76	6	319.9	259.5	130.5	644.9
145.00				585.7	
146.03	6	345.1	244.5	270.9	606.2
147.00				421.9	
148.04	6	434.6	224.3	290.0	686.9
149.00				345.3	
150.00				364.5	
150.75	6	370.1	259.4	194.3	636.8
152.00				426.1	
153.02	6	363.3	251.8	285.8	639.0
154.00				566.5	
155.04	6	441.9	220.5	345.3	670.6
156.00				402.7	
157.00				217.7	
157.72	6	411.4	225.5	126.2	642.4
159.00				5.0	
160.07	6	359.1	241	215.6	599.7
161.00				383.6	
162.06	6	307.5	241.3	160.3	590.7

Table C-8. Transfer data for Train 2 Fermentor 4. Red indicates data was estimated or interpolated based on other data.

Train 2 – Fermentor 4									
Day	**pH**	**Liquid removed**	**Cake added**	**Cake removed**	**Liquid added**	**Gas**	**Total ferm.**	***M* (cake removed)**	***I* (cake removed)**
		g	g	g	mL	cm^3	g	0	0
0.00	5.5	188	64.5	26.3	175		449.8	0.800	
1.78	5.5	199.4	110	79.1	175	94.3	468.5	0.800	
3.89	6	184.6	140.8	129.1	175	64.6	472.9	0.800	
6.00	6	173.8	197.7	196.7	175	77.3	473.0	0.800	
8.02	6	181.6	175.4	166	175	115.6	472.2	0.800	
10.80	6	181.2	220.3	211	175	60.3	471.9	0.800	
12.98	6	156.8	228.6	245.6	175	143.3	473.8	0.800	
14.99	6.5	164.7	221.5	234.8	175	53.9	478.0	0.800	
17.79	6	162.6	199.2	207.7	175	26.3	471.1	0.800	
20.01	6	155.2	213.9	162.3	175	487.8	403.6	0.800	
22.00	6	187.8	243.8	311.6	175	19.9	577.2	0.800	
24.78	6	165.2	242	248.2	175	217.7	490.7	0.800	
26.95	6.5	151.5	211.4	252.3	300	192.2	492.4	0.800	
29.02	6	249.8	233.4	269.6	300	45.4	594.4	0.800	
31.70	6	254.3	221	272.5	300	764.3	605.8	0.800	
33.32	6	257.6	226.8	243.3	300	621.8	592.5	0.800	
36.19	6	277.7	263	263	300	409.1	615.0	0.800	
38.77	6	290.3	290.1	290.1	300	528.2	640.5	0.800	
40.00						164.5		0.800	
40.87	6	279.4	259.4	302.1	300	283.0	659.2	0.800	
42.00						179.4		0.800	
43.12	6	296.3	255.6	250.1	300	723.9	633.5	0.800	
44.00						292.1		0.800	
45.00						645.2		0.800	
45.74	6	305.9	262.5	251.4	300	315.5	636.5	0.800	
47.00						349.6		0.800	
48.01	6	329	245.3	216.8	300	568.7	634.6	0.800	
49.00						196.4		0.800	
50.12	6	318.1	244.7	219.3	300	536.8	625.4	0.800	
51.00						383.6		0.800	
52.00						524.0		0.800	
52.89	6	309.9	275.6	234.9	300	179.4	626.0	0.839	0.277
54.00						283.0			
55.00						319.8			

Table C-8. Continued.

Train 2 – Fermentor 4									
Day	**pH**	**Liquid removed**	**Cake added**	**Cake removed**	**Liquid added**	**Gas**	**Total ferm.**	***M* (cake removed)**	***I* (cake removed)**
		g	g	g	mL	cm^3	g	0	0
56.00						211.3			
57.05	6	441	160.1	68.2	300	196.4	649.1	0.772	0.266
58.00						328.3			
59.00						43.3			
59.75	6	289.9	204.3	206.8	300	198.6	592.4	0.781	0.301
61.00						356.0			
61.96	6	326.4	228.4	184.5	300	234.7	592.4	0.805	0.277
64.82	6	324.9	234.7	184.6	300	232.6	601.0	0.834	0.276
66.00						319.8			
66.72	6	352.8	227.1	188.1	300	217.7	619.5	0.819	0.269
68.00						436.8			
68.73	6	305	198.1	192.5	300	241.1	599.4	0.816	0.280
71.00						751.6			
71.78	6	321	187.1	159.5	300	262.4	593.4	0.820	0.270
73.00						366.6			
73.78	6	290.2	214.3	221.4	300	190.0	597.3	0.839	0.262
75.00						302.8			
76.03	6	326.7	219.2	184.7	300	228.3	595.3	0.829	0.265
77.00						224.1			
78.08	6	319.7	153.7	129.7	300	375.1	595.7	0.830	0.284
79.00						390.0			
80.00						330.4			
80.74	6	305.1	210.9	199.8	300	222.0	594.0	0.837	0.260
82.00						434.7			
83.03	6	342.8	151.5	101.8	300	296.4	593.1	0.803	0.278
84.00						283.0			
85.08	6	313.1	188.6	169.8	300	302.8	594.3	0.824	0.278
86.00						338.9			
87.00						285.8			
87.76	6	328.3	198.2	162.5	300	156.0	592.6	0.811	0.231
89.00						385.7			
90.06	6	305.6	208.9	194.5	300	279.4	591.2	0.810	0.343
91.00						179.4			
92.08	6	353.8	191.7	128.1	300	298.5	590.2	0.799	0.307
93.00						343.2			
94.00						273.0			

Table C-8. Continued.

Train 2 – Fermentor 4									
Day	**pH**	**Liquid removed**	**Cake added**	**Cake removed**	**Liquid added**	**Gas**	**Total ferm.**	***M* (cake removed)**	***I* (cake removed)**
		g	g	g	mL	cm^3	g	0	0
94.73	6	314.6	193.5	171.2	300	162.4	592.3	0.808	0.353
96.00						202.8			
97.09	6	298.8	207.1	192.4	300	219.8	584.1	0.819	0.341
98.00						243.2			
99.10	6	325.7	220.2	187.5	300	194.3	593.0	0.819	0.330
100.00						375.1			
101.00						224.1			
101.73	6	324.4	193.8	163.7	300	143.3	594.3	0.809	0.314
103.00						345.3			
104.08	6	293.7	181.7	173.2	300	283.0	588.9	0.828	0.329
105.00						275.1			
106.10	6	320.4	193.1	172	300	241.1	599.3	0.823	0.379
107.00						377.2			
108.00						232.6			
108.75	6.1	365.4	200.8	129.9	300	102.8	594.5	0.791	0.371
110.00						283.0			
111.06	6	300	213.6	207.4	300	151.8	593.8	0.826	0.324
112.00						234.7			
113.08	6	326.1	191	159.4	300	256.0	594.5	0.828	0.273
114.00						236.8			
115.00						192.2			
115.76	6.1	320.9	210.8	189.4	300	94.3	599.5	0.813	0.322
117.00						245.4			
118.08	6	329.8	213.8	184.5	300	185.8	600.5	0.815	0.301
119.00						222.0			
120.11	6	336	189.7	138.8	300	145.4	585.0	0.832	0.314
121.00						283.6			
122.00						215.6			
122.76	6.1	328	213.4	186.9	300	100.7	601.5	0.815	0.342
124.00						243.2			
125.09	6.1	319.7	207.5	179.7	300	166.7	591.9	0.822	0.280
126.00						251.7			
127.10	6	349.1	157.2	101.9	300	164.5	593.8	0.797	0.322
128.00						292.1			
129.00						192.2			
129.73	6	332.3	168	128	300	96.5	592.3	0.794	0.301

Table C-8. Continued.

Train 2 – Fermentor 4									
Day	**pH**	**Liquid removed**	**Cake added**	**Cake removed**	**Liquid added**	**Gas**	**Total ferm.**	***M* (cake removed)**	***I* (cake removed)**
		g	g	g	mL	cm^3	g	0	0
131.00						294.3			
132.06	6	333.3	183.7	147.3	300	181.5	596.9	0.773	0.326
133.00						209.2			
134.05	6	330.6	206.1	169.4	300	151.8	593.9	0.787	0.330
135.00						162.4			
136.00						170.9			
136.73	6	328.7	207.2	174.2	300	85.8	595.7	0.793	0.298
138.00						177.3			
139.10	6	337.8	195	145.9	300	164.5	588.7	0.800	0.316
140.00						228.3			
141.03	6	356.3	154.2	92.1	300	128.4	594.2	0.786	0.271
142.00						292.1			
143.00						177.3			
143.76	6	277.5	246.5	235.2	300	79.4	590.3	0.802	0.271
145.00						290.0			
146.03	6	370.9	186.2	134.7	300	117.7	619.4	0.810	0.293
147.00						241.1			
148.04	6	346.8	175	120.1	300	122.0	591.9	0.764	0.363
149.00						217.7			
150.00						126.2			
150.75	6	311.7	186.5	168.5	300	88.0	593.7	0.820	0.345
152.00						194.3			
153.02	6	349.6	196.6	196.4	300	168.8	649.4	0.779	0.301
154.00						296.4			
155.04	6	351.5	149.2	96	300	181.5	598.3	0.775	0.274
156.00						270.9			
157.00						151.8			
157.72	6	307.2	154.5	138.8	300	107.1	591.5	0.756	0.383
159.00						5.0			
160.07	6	303.7	161.8	152.8	300	170.9	594.7	0.763	0.299
161.00						194.3			
162.06	6	274.5	208.4	204.5	300	141.1	570.6	0.825	0.357

Table C-9. Transfer data for Train 3 Fermentor 1. Red indicates data was estimated or interpolated based on other data.

Train 3 – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
0.00	6.5	99.8	35		449.6	0.960	0.350
1.75	6	89.3	35	119.9	464.0	0.960	0.350
3.90	6	105.1	35	213.4	494.7	0.960	0.350
5.97	6.5	61.5	35	209.2	450.7	0.960	0.350
8.02	6	56.2	35	243.2	459.1	0.960	0.350
10.80	6	19.7	35	285.8	422.2	0.960	0.350
12.92	6.5	18.7	35	239.0	421.5	0.960	0.350
14.97	6	41.3	35	194.3	442.3	0.960	0.350
17.77	6	3.6	35	79.4	409.5	0.960	0.350
20.00	6.5	13.1	35	134.7	406.4	0.960	0.350
21.98	6.5	10.7	35	128.4	393.7	0.960	0.350
24.77	6	3.1	35	102.8	258.2	0.960	0.350
26.94	7	1.2	35	122.0	255.8	0.960	0.350
28.29	0	0	35	445.3	230.9	0.960	0.350
31.67	0	0	35	241.1	243.1	0.960	0.350
34.07	0	0	35	85.8	271.5	0.960	0.350
36.19	0	0	35	62.4	287.9	0.960	0.350
38.72	0	0	35	341.1	305.9	0.960	0.350
40.00				30.5			
40.86	6.5	27.2	35	87.5	345.1	0.960	0.350
42.00				49.7			
43.10	6.5	68.8	35	77.3	385.6	0.960	0.350
44.00				107.1			
45.00				64.6			
45.73	6.5	62.1	35	47.5	364.1	0.960	0.350
47.00				143.3			
48.01	6.5	30.5	35	26.3	458.9	0.960	0.350
49.00				79.4			
50.08	6.5	34.9	35	60.3	359.5	0.960	0.350
51.00				117.7			
52.00				64.6			
52.89	6.5	128.2	35	19.9	461.2	0.960	0.350
54.00				168.8			
55.00				47.5			

Table C-9. Continued.

Train 3 – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
56.00				47.5			
57.05	6.5	92.8	35	60.3	425.2	0.960	0.350
58.00				102.8			
59.00				53.9			
59.72	6.5	53.5	35	62.4	417.7	0.960	0.350
61.00				117.7			
61.95	7	121.2	35	85.8	447.1	0.960	0.350
64.82	6.5	171.3	35	90.1	498.4	0.960	0.350
66.00				73.1			
66.69	6.5	132.7	35	75.2	463.3	0.960	0.350
68.00				160.3			
68.70	6.5	118.7	35	58.2	433.3	0.960	0.350
71.00				179.4			
71.77	6.5	147.2	35	43.3	470.5	0.960	0.350
73.00				177.3			
73.75	6.5	113	35	53.9	441.0	0.960	0.350
75.00				70.9			
76.00	6.5	179	35	83.7	489.3	0.960	0.350
77.00				119.9			
78.07	6.5	159.1	35	58.2	477.6	0.960	0.350
79.00				132.6			
80.00				62.4			
80.73	6.5	147.4	35	36.9	467.5	0.960	0.350
82.00				56.0			
83.01	6.5	132.4	35	79.4	468.7	0.959	0.300
84.00				87.5			
85.07	6.5	149.8	35	83.7	463.1	0.965	0.390
86.00				107.1			
87.00				62.4			
87.74	6	177.5	35	39.0	493.6	0.966	0.362
89.00				132.6			
90.04	6.5	131.6	35	49.7	479.9	0.948	0.460
91.00				151.8			
92.07	6	157.1	35	45.4	483.5	0.969	0.478
93.00				145.4			
94.00				56.0			

Table C-9. Continued.

Train 3 – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
94.72	6.5	207.1	35	17.8	525.0	0.968	1.078
96.00				115.6			
97.08	6.5	145.7	35	128.4	467.9	0.960	0.750
98.00				60.3			
99.08	6	149.7	35	92.2	478.8	0.964	0.842
100.00				126.2			
101.00				94.3			
101.72	6	163	35	49.7	472.7	0.949	0.905
103.00				234.7			
104.07	6	146	35	87.5	469.5	0.948	0.610
105.00				113.5			
106.07	6	170.7	35	49.7	487.8	0.957	0.508
107.00				151.8			
108.00				32.7			
108.73	6.5	158.5	35	28.4	490.0	0.965	0.600
110.00				86.0			
111.02	6	144.2	35	51.8	469.8	0.970	0.632
112.00				79.4			
113.06	6	208	35	92.2	534.4	0.962	0.450
114.00				173.0			
115.00				49.7			
115.73	6.5	159.2	35	43.3	506.1	0.976	0.647
117.00				181.5			
118.06	6	136	35	56.0	469.3	0.963	0.604
119.00				107.1			
120.07	6	149.7	35	70.9	490.6	0.962	0.683
121.00				81.6			
122.00				62.4			
122.75	6.1	203.5	35	36.9	524.5	0.956	0.581
124.00				149.6			
125.05	6	168.7	35	66.7	477.6	0.962	0.180
126.00				226.2			
127.09	6	191.2	35	62.4	506.8	0.965	0.444
128.00				179.4			
129.00				109.2			
129.72	6.1	176.5	35	45.4	501.4	0.963	0.618

Table C-9. Continued.

Train 3 – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
131.00				177.3			
132.04	6	152.7	35	126.2	495.0	0.953	0.387
133.00				183.7			
134.04	6	163.0	35	166.7	500.4	0.964	0.858
135.00				292.1			
136.00				85.8			
136.73	6.5	143.2	35	32.7	461.9	0.957	0.607
138.00				153.9			
139.09	6	115.5	35	183.7	467.1	0.958	0.918
140.00				234.7			
141.02	6	196.0	35	145.4	509.8	0.961	0.856
142.00				307.0			
143.00				92.2			
143.70	6.5	166.0	35	47.5	516.7	0.959	0.623
145.00				245.4			
146.01	6.5	144.8	35	73.1	480.0	0.955	0.456
147.00				149.6			
148.03	6.5	163.3	35	90.1	530.8	0.959	0.155
149.00				136.9			
150.00				75.2			
150.71	6.5	146.9	35	68.8	470.9	0.958	0.498
152.00				128.4			
153.01	6	115.2	35	124.1	512.2	0.968	0.644
154.00				213.4			
155.04	6	96.7	35	132.6	450.3	0.962	0.541
156.00				226.2			
157.00				122.0			
157.72	6	181.0	35	77.3	549.0	0.959	0.639
159.00				5.0			
160.05	6	168.4	35	279.4	532.7	0.871	0.852
161.00				241.1			
162.05	6.5	178.5	35	122.0	537.9	0.963	0.931

Table C-10. Transfer data for Train 3 Fermentor 2. Red indicates data was estimated or interpolated based on other data.

Train 3 – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
0.00	6	170.6	84.8		449.8
1.75	6	201.1	109.7	62.4	484.9
3.90	6	166.6	154.6	98.6	503.3
5.97	6.5	128.3	124.2	111.4	446.1
8.02	6	108	137.9	156.0	442.3
10.80	6	124.5	137.5	130.5	461.5
12.92	6	154.5	137.8	196.4	504.3
14.97	6	109.4	136	62.4	448.9
17.77	6	112.2	140.9	88.0	471.0
20.00	6.5	82.6	35	96.5	421.4
21.98	6.5	82.6	218	147.5	420.6
24.77	6	55.5	90.1	145.4	415.9
26.94	6.5	34.6	89.6	145.4	387.0
28.29	6.5	47.9	65.9	148.0	331.8
31.67	6.5	74.3	78.1	273.0	435.5
34.07	6.5	92.9	106.5	213.4	486.3
36.19	6	102.3	122.9	196.4	500.5
38.72	6	148.9	140.9	185.8	495.8
40.00				115.6	
40.86	6	187.8	152.9	148.0	537.7
42.00				117.7	
43.10	6.5	174	151.8	85.8	529.1
44.00				111.4	
45.00				119.9	
45.73	6.5	160.7	37	53.9	505.0
47.00				228.3	
48.01	6.5	173.6	263.4	58.2	501.6
49.00				119.9	
50.08	6.5	264.2	159.6	90.1	652.4
51.00				128.4	
52.00				88.0	
52.89	6.5	231.1	168	49.7	595.4
54.00				183.7	
55.00				60.3	

Table C-10. Continued.

Train 3 – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
56.00				62.4	
57.05	6.5	222.4	167.4	73.1	557.1
58.00				192.2	
59.00				41.2	
59.72	6.5	257.8	199.2	109.2	612.8
61.00				128.4	
61.95	6.5	304.8	160.9	85.8	656.0
64.82	6.5	265.5	162.1	70.9	611.5
66.00				102.8	
66.69	6.5	243.3	165.6	79.4	565.3
68.00				162.4	
68.70	6.5	276.1	149.6	45.4	611.1
71.00				230.5	
71.77	6.5	247	158.3	83.7	584.8
73.00				239.0	
73.75	6.5	294.2	163	92.2	646.9
75.00				128.4	
76.00	6	281.5	145.3	100.7	631.0
77.00				190.0	
78.07	6	271.6	153.5	102.8	608.6
79.00				222.0	
80.00				100.7	
80.73	6.5	278.6	155.1	79.4	630.5
82.00				132.6	
83.01	6.5	266.6	171.3	111.4	608.0
84.00				148.0	
85.07	6.5	296.6	148.3	115.6	644.0
86.00				194.3	
87.00				96.5	
87.74	6	287.1	151.1	11.4	626.8
89.00				239.0	
90.04	6.5	286.7	183.3	141.1	640.3
91.00				236.8	
92.07	6	327.3	161.4	107.1	670.0
93.00				168.8	

Table C-10. Continued.

Train 3 – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
94.00				119.9	
94.72	6.5	281.6	152.9	92.2	624.8
96.00				192.2	
97.08	6	278.8	157.2	183.7	624.0
98.00				73.1	
99.08	6	278.5	164.1	94.3	624.3
100.00				187.9	
101.00				109.2	
101.72	6	276.8	144.7	77.3	626.6
103.00				304.9	
104.07	6	289.5	258.5	148.0	657.5
105.00				183.7	
106.07	6	293.7	152.1	115.6	625.7
107.00				192.2	
108.00				130.5	
108.73	6.1	305	166.5	90.1	659.5
110.00				270.9	
111.02	6	335.9	187.6	122.0	664.6
112.00				141.1	
113.06	6	309.9	161.4	113.5	640.9
114.00				219.8	
115.00				19.9	
115.73	6.5	279.7	181.9	105.0	622.2
117.00				258.1	
118.06	6	295.2	168.3	153.9	638.7
119.00				39.0	
120.07	6	328	175.9	124.1	665.7
121.00				168.8	
122.00				117.7	
122.75	6.1	290.7	156	73.1	636.4
124.00				236.8	
125.05	6	312.3	143.9	136.9	657.8
126.00				258.1	
127.09	6	309.8	150.6	156.0	648.6
128.00				198.6	
129.00				136.9	

Table C-10. Continued.

Train 3 – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
129.72	6.1	305.9	159.9	83.7	655.8
131.00				245.4	
132.04	6	307.4	177.3	164.5	654.4
133.00				230.5	
134.04	6	267.1	172.4	177.3	616.8
135.00				262.4	
136.00				111.4	
136.73	6.5	284.2	153.7	64.6	619.4
138.00				379.4	
139.09	6	313.7	186.6	196.4	661.9
140.00				234.7	
141.02	6	321.8	148.8	141.1	643.9
142.00				249.6	
143.00				90.1	
143.70	6	288.9	185.7	51.8	619.5
145.00				179.4	
146.01	6	337.2	170.2	115.6	669.6
147.00				185.8	
148.03	6	274.2	202.5	81.6	602.8
149.00				141.1	
150.00				81.6	
150.71	6	320.6	159	60.3	651.6
152.00				226.2	
153.01	6	254	232	83.7	614.9
154.00				245.4	
155.04	6	353.8	188.6	122.0	689.7
156.00				245.4	
157.00				109.2	
157.72	6	340	203	68.8	677.3
159.00				5.0	
160.05	6	369.4	199.3	166.7	702.5
161.00				217.7	
162.05	6	293.6	194.4	134.7	619.0

Table C-11. Transfer data for Train 3 Fermentor 3. Red indicates data was estimated or interpolated based on other data.

Train 3 – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
0.00	6	195.1	64		449.5
1.75	6	206.3	93.5	119.9	482.3
3.90	6.5	191.5	161.3	115.6	473.5
5.97	6.5	165.5	142	185.8	466.2
8.02	6.5	152.1	172.2	132.6	456.1
10.80	5.5	208.6	174.5	796.2	482.9
12.92	6	160.3	187.6	343.2	491.1
14.97	6	179.4	175.5	239.0	500.6
17.77	6	129	199.7	413.4	453.2
20.00	6	124	167.1	366.6	365.6
21.98	6.5	123.6	256	358.1	455.7
24.77	6	96.3	150.5	458.1	454.3
26.94	6	138.3	142	458.1	473.6
28.29	6.5	132.5	149.8	279.4	461.6
31.67	6	207.4	139.3	583.5	547.0
34.07	6	209.8	200	470.8	572.6
36.19	6	199	221.1	438.9	575.7
38.72	6	244.8	187.8	628.2	613.9
40.00				268.7	
40.86	6	237.2	202.8	470.0	592.2
42.00				151.8	
43.10	6	202	210.9	287.9	553.0
44.00				247.5	
45.00				434.7	
45.73	6	209.1	81.3	247.5	583.7
47.00				526.1	
48.01	6	315.5	253.4	362.3	604.6
49.00				211.3	
50.08	6.5	301.7	247.8	664.4	654.4
51.00				243.2	
52.00				251.7	
52.89	6	267.5	232.3	158.1	675.0
54.00				898.3	
55.00				268.7	
56.00				287.9	

Table C-11. Continued.

Train 3 – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
57.05	6	314.4	202.1	579.3	633.5
58.00				204.9	
59.00				26.3	
59.72	6	368.1	254.2	247.5	685.0
61.00				200.7	
61.95	6.5	317	212.1	145.4	683.8
64.82	6.5	269.3	208.1	190.0	605.8
66.00				134.7	
66.69	6.5	315.8	187.6	198.6	638.2
68.00				279.4	
68.70	6	287.7	184.6	262.4	626.6
71.00				732.4	
71.77	6	354.2	196.1	481.4	628.5
73.00				519.7	
73.75	6	337.7	215.7	332.6	642.5
75.00				262.4	
76.00	6	314.3	194.8	472.9	632.7
77.00				249.6	
78.07	6	335.5	190.5	689.9	621.8
79.00				366.6	
80.00				596.3	
80.73	6	315.5	207	356.0	619.5
82.00				417.6	
83.01	6.5	351.3	212.7	564.4	636.2
84.00				470.0	
85.07	6	331.4	195.7	628.2	659.7
86.00				302.8	
87.00				615.4	
87.74	6	349	190.8	455.9	599.3
89.00				532.5	
90.04	6	374.6	236.9	730.3	648.0
91.00				470.0	
92.07	6	331.7	205.1	717.5	665.5
93.00				373.0	
94.00				494.2	
94.72	6	335.5	196.1	351.7	638.6

Table C-11. Continued.

Train 3- Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
96.00				321.9	
97.08	6	332.8	202.4	658.0	628.8
98.00				217.7	
99.08	6	333.1	209.9	409.1	634.6
100.00				292.1	
101.00				324.1	
101.72	6	365.9	194.5	402.7	660.4
103.00				564.4	
104.07	6	330	226.5	470.0	618.9
105.00				349.6	
106.07	6	368.7	184.1	740.9	652.1
107.00				485.7	
108.00				675.0	
108.73	6.1	374.1	221	317.7	638.7
110.00				600.6	
111.02	6	348.8	216.3	694.1	634.6
112.00				307.0	
113.06	6	328	192.4	749.5	637.0
114.00				419.8	
115.00				670.8	
115.73	6.1	346.8	224.4	341.1	614.0
117.00				549.5	
118.06	6	369.5	211.8	677.1	663.3
119.00				419.8	
120.07	6	344	213.6	577.2	642.2
121.00				353.8	
122.00				617.6	
122.75	6.1	365.6	201.7	402.7	654.7
124.00				398.5	
125.05	6	353.2	189.4	600.6	640.5
126.00				517.6	
127.09	6	363.8	189.4	617.6	632.4
128.00				366.6	
129.00				421.9	
129.72	6.1	357.1	209.8	377.2	646.9
131.00				377.2	

Table C-11. Continued.

Train 3 – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
132.04	6	324.5	224.3	443.2	632.7
133.00				336.8	
134.04	6	324.6	222.1	264.5	652.4
135.00				392.1	
136.00				345.3	
136.73	6	378.3	188.9	353.8	660.3
138.00				549.5	
139.09	6	350.7	234.8	660.1	625.1
140.00				379.4	
141.02	6	324.5	170.9	243.2	642.4
142.00				341.1	
143.00				207.1	
143.70	6	376.4	216.3	192.2	665.1
145.00				230.5	
146.01	6	306.8	202.6	124.1	620.3
147.00				258.1	
148.03	6	352.6	231.1	247.5	627.7
149.00				185.8	
150.00				207.1	
150.71	6	322.5	190	345.3	650.5
152.00				451.7	
153.01	6	386.5	283.3	734.6	673.3
154.00				321.9	
155.04	6	380.9	224.5	436.8	701.4
156.00				360.2	
157.00				222.0	
157.72	6	405	240.3	300.7	690.7
159.00				5.0	
160.05	6	349.8	232.4	364.5	659.9
161.00				347.4	
162.05	6	352.6	219.8	409.1	647.4

Table C-12. Transfer data for Train 3 Fermentor 4. Red indicates data was estimated or interpolated based on other data.

Train 3 – Fermentor 4									
Day	**pH**	**Liquid removed**	**Cake added**	**Cake removed**	**Liquid added**	**Gas**	**Total ferm.**	***M* (cake removed)**	***I* (cake removed)**
		g	g	g	mL	cm^3	g	0	0
0.00	5.5	190.1	47.3	7.1	175		449.9	0.800	
1.75	5.5	188.6	98.4	83.1	175	81.6	473.3	0.800	
3.90	6.5	166.1	172.2	151.2	175	83.7	445.1	0.800	
5.97	6.5	189.2	166.7	148.3	175	49.7	470.8	0.800	
8.02	6.5	174.6	200.2	193	175	98.6	467.4	0.800	
10.80	6	195.9	172.8	143.1	175	879.2	466.2	0.800	
12.92	6	205.4	242.4	207.3	175	481.4	470.3	0.800	
14.97	6	158.4	220.7	256	175	379.4	493.7	0.800	
17.77	6	172.2	246.6	221.3	175	490.0	471.3	0.800	
20.00	6	157.2	232.7	270.5	175	302.8	495.0	0.800	
21.98	6	109.7	256.2	283	175	502.7	465.7	0.800	
24.77	6	178.4	178	197.3	175	830.3	497.7	0.800	
26.94	6	170.5	201.3	255.7	300	560.1	524.9	0.800	
28.29	6.5	265.7	202.9	227.4	300	198.6	590.2	0.800	
31.67	5.5	276.7	202.9	218.3	300	983.4	592.1	0.800	
34.07	6	279.1	286.8	241.3	300	1021.7	595.6	0.800	
36.19	6	293.2	303.8	363.5	300	526.1	652.9	0.800	
38.72	6	297.7	280.9	299.1	300	653.7	676.4	0.800	
40.00						228.3		0.800	
40.86	6	260.1	278.4	318.7	300	450.0	655.7	0.800	
42.00						147.5		0.800	
43.10	6	279.2	273.5	340.5	300	764.3	646.2	0.800	
44.00						381.5		0.800	
45.00						813.3		0.800	
45.73	6	311.8	179.9	215.2	300	300.7	647.1	0.800	
47.00						534.6		0.800	
48.01	6	311.1	204.8	189	300	307.0	595.3	0.800	
49.00						317.7		0.800	
50.08	6	332	273	229.3	300	381.5	588.3	0.800	
51.00						377.2		0.800	
52.00						679.3		0.800	
52.89	6	310.5	329.3	258.6	300	277.3	651.3	0.835	0.252
54.00						594.2			
55.00						404.9			

Table C-12. Continued.

Train 3 – Fermentor 4									
Day	**pH**	**Liquid remove**	**Cake adde**	**Cake remove**	**Liqui d**	**Gas**	**Total ferm.**	***M* (cake removed**	***I* (cake removed**
		g	g	g	mL	cm^3	g	0	0
56.00						211.3			
57.05	6	385.7	241.2	232.9	300	302.8	700.0	0.797	0.287
58.00						566.5			
59.00						39.0			
59.72	6	341.4	241.7	194.5	300	266.6	614.3	0.827	0.283
61.00						321.9			
61.95	6	294.1	283.4	226	300	338.9	609.3	0.844	0.286
64.82	6	324	250.5	252.3	300	296.4	663.4	0.836	0.259
66.00						173.0			
66.69	6.5	334.8	234	213.3	300	266.6	628.3	0.845	0.256
68.00						555.9			
68.70	6	334.3	247.5	187.9	300	241.1	607.7	0.837	0.265
71.00						836.7			
71.77	6	351.2	194.4	163.8	300	332.6	620.6	0.830	0.260
73.00						796.2			
73.75	6	322	220.6	191.5	300	260.2	592.9	0.826	0.302
75.00						589.9			
76.00	6	322.4	230	183	300	472.9	592.2	0.830	0.296
77.00						500.6			
78.07	6	333.6	200.8	174.4	300	632.5	607.2	0.835	0.247
79.00						617.6			
80.00						536.8			
80.73	6	327.2	215.8	184.8	300	243.2	596.2	0.833	0.299
82.00						541.0			
83.01	6	355.4	202.3	138.4	300	281.5	591.5	0.825	0.267
84.00						450.0			
85.07	6	305.9	244.9	202.5	300	562.3	592.4	0.823	0.287
86.00						332.6			
87.00						664.4			
87.74	6	357.8	165.1	128.5	300	328.3	621.2	0.838	0.214
89.00						634.6			
90.04	6	335.1	194.8	152.3	300	383.6	592.6	0.799	0.332
91.00						577.2			
92.07	6	347.7	256.1	164.5	300	362.3	594.6	0.814	0.328
93.00						555.9			
94.00						390.0			

Table C-12. Continued.

Train 3 – Fermentor 4									
Day	**pH**	**Liquid remove**	**Cake adde**	**Cake remove**	**Liqui d**	**Gas**	**Total ferm.**	***M* (cake removed**	***I* (cake removed**
		g	g	g	mL	cm^3	g	0	0
94.72	6	344.1	223.2	202.7	300	232.6	629.5	0.817	0.313
96.00						485.7			
97.08	6	339.1	216.1	167.8	300	438.9	590.0	0.827	0.420
98.00						460.2			
99.08	6	357.4	219.9	151	300	317.7	591.5	0.809	0.421
100.0						356.0			
101.0						396.4			
101.7	6	331.9	213	171.5	300	226.2	590.4	0.827	0.319
103.0						630.3			
104.0	6	329.7	210.8	180.4	300	450.0	599.3	0.823	0.320
105.0						532.5			
106.0	6	350.8	191.5	134.8	300	462.3	594.1	0.815	0.357
107.0						500.6			
108.0						666.5			
108.7	6.1	317.3	182.8	155.3	300	170.9	589.8	0.818	0.330
110.0						636.7			
111.0	6	323.9	201.7	172.3	300	315.5	594.5	0.822	0.326
112.0						455.9			
113.0	6	323.6	225.2	188.6	300	521.9	590.5	0.816	0.307
114.0						628.2			
115.0						428.3			
115.7	6.1	340.7	182.1	138	300	232.6	596.6	0.832	0.385
117.0						634.6			
118.0	6	339.1	215.9	168.8	300	383.6	592.0	0.820	0.343
119.0						713.3			
120.0	6	355.6	216.3	156	300	362.3	595.3	0.816	0.327
121.0						598.4			
122.0						373.0			
122.7	6.1	350.5	206.1	145.4	300	194.3	589.8	0.809	0.460
124.0						589.9			
125.0	6	341.1	200.7	154.2	300	334.7	594.6	0.824	0.260
126.0						598.4			
127.0	6	365.7	182	108.2	300	315.5	591.9	0.806	0.355
128.0						689.9			
129.0						358.1			
129.7	6	329.9	206.7	173.9	300	196.4	597.1	0.824	0.428

Table C-12. Continued.

Train 3 – Fermentor 4									
Day	**pH**	**Liquid remove**	**Cake adde**	**Cake remove**	**Liqui d**	**Gas**	**Total ferm.**	***M* (cake removed**	***I* (cake removed**
		g	g	g	mL	cm^3	g	0	0
131.0						526.1			
132.0	6	337.5	229.3	186.2	300	324.1	694.4	0.821	0.324
133.0						596.3			
134.0	6	344.7	247.3	173	300	347.4	599.9	0.825	0.292
135.0						662.2			
136.0						334.7			
136.7	6	341.1	194.9	177.1	300	194.3	623.3	0.831	0.245
138.0						624.0			
139.0	6	313.5	195	166.1	300	424.0	584.6	0.829	0.311
140.0						532.5			
141.0	6	374.6	212.8	130.3	300	298.5	592.1	0.797	0.298
142.0						347.4			
143.0						222.0			
143.7	6	308.9	208	192.3	300	136.9	593.2	0.831	0.360
145.0						475.1			
146.0	6	328	230	183.5	300	300.7	592.6	0.828	0.379
147.0						196.4			
148.0	6	322.3	195.4	175	300	500.6	601.9	0.831	0.407
149.0						326.2			
150.0						324.1			
150.7	6	361	218	153.7	300	219.8	596.7	0.798	0.358
152.0						638.8			
153.0	6	323.5	206.2	174.7	300	347.4	592.0	0.820	0.347
154.0						698.4			
155.0	6	374	242.7	141	300	538.9	593.6	0.796	0.413
156.0						721.8			
157.0						313.4			
157.7	6	325.5	207.6	197.5	300	241.1	615.4	0.817	0.444
159.0						5.0			
160.0	6	345.9	231.1	174.3	300	404.9	599.2	0.822	0.263
161.0						624.0			
162.0	6	323.7	220.6	175	300	309.2	578.1	0.842	0.322

Table C-13. Transfer data for Train 4 Fermentor 1. Red indicates data was estimated or interpolated based on other data.

Train 4 – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
0.00	6.5	84.6	35		449.7	0.960	0.329
1.82	6	78.3	35	96.5	450.3	0.960	0.329
3.98	6	97.6	35	202.8	509.7	0.960	0.329
6.03	6.5	47.9	35	128.4	462.2	0.960	0.329
8.07	6	94.2	35	149.6	488.9	0.960	0.329
10.87	6	34.4	35	219.8	438.5	0.960	0.329
12.98	6	30.7	35	394.2	433.9	0.960	0.329
15.06		0	35	136.9	373.8	0.960	0.329
17.83	6.5	1.4	35	53.9	407.2	0.960	0.329
19.34	6.5	0	35	59.0	330.8	0.960	0.329
22.05	7	0	35	107.1	292.9	0.960	0.329
24.83	6.5	3.7	35	162.4	250.7	0.960	0.329
26.34		0	35	111.4	261.9	0.960	0.329
29.07		0	35	141.1	250.9	0.960	0.329
31.74		0	35	164.5	247.9	0.960	0.329
34.15		0	35	100.7	212.7	0.960	0.329
36.27		0	35	51.8	304.0	0.960	0.329
38.79		0	35	53.9	300.3	0.960	0.329
40.00				30.5			
40.92		0	35	59.0	199.8	0.960	0.329
42.00				7.1			
42.34		0	35	15.6	206.1	0.960	0.329
44.00				59.0			
45.00				79.4			
45.80		0	35	28.4	296.4	0.960	0.329
47.00				34.8			
48.07		0	35	26.3	297.6	0.960	0.329
49.00				134.7			
50.00	7	10	35	130.5	306.1	0.960	0.329
51.00				128.4			
52.00				59.0			
52.96		0	35	59.0	320.9	0.960	0.329
54.00				96.5			
55.00				19.9			
56.00				30.5			

Table C-13. Continued.

Train 4 – Fermentor 1							
Day	**pH**	**Liquid remove**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
57.11	6.5	27.8	35	36.9	339.6	0.960	0.329
58.00				96.5			
59.00				32.7			
59.79	7	51.2	35	22.0	368.1	0.960	0.329
61.00				128.4			
62.02	6.5	42.2	35	5.0	357.3	0.960	0.329
64.89	6.5	100.1	35	11.4	412.5	0.960	0.329
66.00				92.2			
66.75	6.5	72.5	35	59.0	372.8	0.960	0.329
68.00				141.1			
68.76	6.5	122.5	35	79.4	423.0	0.960	0.329
71.00				117.7			
71.83	6.5	139.8	35	28.4	440.4	0.960	0.329
73.00				73.1			
73.82	7	105.7	35	9.3	431.2	0.960	0.329
75.00				117.7			
76.07	6.5	73.3	35	83.7	394.6	0.960	0.329
77.00				92.2			
78.13	6.5	104	35	92.2	413.3	0.960	0.329
79.00				107.1			
80.00				36.9			
80.79	6.5	46.8	35	9.3	382.9	0.960	0.329
82.00				134.7			
83.08	6.5	72.6	35	88.0	368.0	0.962	0.279
84.00				59.0			
85.12	6.5	61	35	73.1	379.3	0.962	0.336
86.00				113.5			
87.00				100.7			
87.81	6.5	46.1	35	15.6	375.9	0.966	0.373
89.00				119.9			
90.12		0	35	53.9	259.1	0.960	0.700
91.00				130.5			
92.12	6.5	63.6	35	45.4	410.4	0.957	0.768
93.00				51.8			
94.00				81.6			
94.78	6.5	72.6	35	9.3	411.1	0.967	0.949

Table C-13. Continued.

Train 4 – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
96.00				64.6			
97.14	6.5	13.2	35	179.4	327.4	0.960	0.850
98.00				70.9			
99.15	6	113.9	35	111.4	417.5	0.960	0.647
100.00				162.4			
101.00				111.4			
101.78	6.5	94.9	35	9.3	442.1	0.936	0.678
103.00				98.6			
104.13	6	89.8	35	59.0	436.1	0.957	0.522
105.00				107.1			
106.14	6	110.1	35	45.4	419.6	0.957	0.512
107.00				98.6			
108.00				36.9			
108.80	6.1	154.2	35	11.4	499.3	0.963	0.806
110.00				122.0			
111.12	6	131.0	35	7.1	482.3	0.968	0.628
112.00				75.2			
113.14	6	115	35	36.9	456.1	0.969	0.213
114.00				102.8			
115.00				49.7			
115.81	6.1	73.6	35	7.1	440.4	0.963	0.710
117.00				117.7			
118.13	6	80.4	35	13.5	416.7	0.969	0.809
119.00				88.0			
120.16	6	144.9	35	19.9	497.6	0.966	0.745
121.00				124.1			
122.00				70.9			
122.81	6.2	76.3	35	5.0	431.4	0.966	0.622
124.00				102.8			
125.13	6.1	171.3	35	79.4	479.6	0.970	0.710
126.00				102.8			
127.15	6	60.6	35	77.3	394.8	0.957	0.710
128.00				109.2			
129.00				28.4			
129.79	6	203.3	35	39.0	508.8	0.972	0.658
131.00				156.0			

Table C-13. Continued.

Train 4 – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
132.10	6	138.5	35	117.7	451.6	0.959	0.907
133.00				122.0			
134.09	6.5	157.5	35	81.6	460.5	0.726	0.945
135.00				102.8			
136.00				49.7			
136.78	6.5	218.3	35	34.8	514.2	0.961	0.655
138.00				187.9			
139.14	6	170.4	35	173.0	478.6	0.965	0.904
140.00				239.0			
141.09	6	174.7	35	51.8	485.2	0.962	0.693
142.00				151.8			
143.00				68.8			
143.82	6	170.9	35	41.2	549.4	0.964	0.655
145.00				190.0			
146.08	6	129.9	35	58.2	465.4	0.961	0.691
147.00				211.3			
148.09	6.5	183.8	35	85.8	518.7	0.960	0.532
149.00				181.5			
150.00				45.4			
150.81	6.5	123.4	35	13.5	484.2	0.961	0.521
152.00				141.1			
153.07	6.5	141.7	35	75.2	483.1	0.955	0.550
154.00				153.9			
155.10	6.5	96	35	30.5	446.8	0.964	0.441
156.00				83.7			
157.00				22.0			
157.78	6.5	135.2	35	30.5	487.6	0.962	0.647
159.00				5.0			
160.13	6.5	129.3	35	102.8	484.5	0.961	0.556
161.00				105.0			
162.10	6.5	149.1	35	62.4	482.9	0.961	0.719

Table C-14. Transfer data for Train 4 Fermentor 2. Red indicates data was estimated or interpolated based on other data.

Train 4 – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
0.00	6	155.6	100.1		449.6
1.82	5.5	214.1	107	66.7	485.7
3.98	6	193.5	147.1	83.7	503.7
6.03	6	144.8	149.3	92.2	479.9
8.07	6	167.3	129.7	58.2	485.6
10.87	6	138.8	139.1	41.2	455.3
12.98	6	76.9	138.2	90.1	417.8
15.06	6.5	113.9	108.8	126.2	488.0
17.83	6	34.1	140.8	77.3	337.0
19.34	6.5	99.8	210.8	96.5	445.8
22.05	6.5	50.9	127.9	83.7	418.3
24.83	6.5	67.4	82	111.4	404.4
26.34	6.5	55.1	96.9	107.1	414.8
29.07	6.5	49.4	85.9	62.0	421.7
31.74	6.5	8.7	82.9	158.1	363.8
34.15	6.5	102.7	47.7	194.3	489.5
36.27	6.5	101.6	139	45.4	484.1
38.79	0	0	135.3	15.6	299.5
40.00				36.9	
40.92	6.5	6.9	34.8	62.0	356.5
42.00				94.3	
42.34	7	100	41.1	15.6	464.5
44.00				51.8	
45.00				88.0	
45.80	6.5	99.4	131.4	436.8	480.2
47.00				139.0	
48.07	6	116.1	132.6	39.0	507.2
49.00				130.5	
50.00	6.5	127.9	131.1	28.4	504.2
51.00				119.9	
52.00				32.7	
52.96	6.5	143.3	155.9	22.0	497.8
54.00				109.2	
55.00				53.9	
56.00				34.8	

Table C-14. Continued.

Train 4 – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
57.11	6	172.6	146.8	15.6	514.7
58.00				109.2	
59.00				45.4	
59.79	6.5	161.8	151.9	32.7	500.6
61.00				109.2	
62.02	6.5	213.3	150.1	34.8	574.7
64.89	6.5	174.3	174.4	56.0	515.9
66.00				105.0	
66.75	6	227.8	135.3	19.9	562.7
68.00				105.0	
68.76	6	242.2	135.5	34.8	568.9
71.00				141.1	
71.83	6.5	234.1	135.6	49.7	571.6
73.00				70.9	
73.82	6.5	202.9	160.5	22.0	566.9
75.00				107.1	
76.07	6.5	217.3	156.3	49.7	539.4
77.00				94.3	
78.13	6	185.8	144.3	39.0	496.6
79.00				128.4	
80.00				28.4	
80.79	6.5	176.3	171.1	15.6	524.9
82.00				119.9	
83.08	6.5	183.5	130.4	64.6	514.0
84.00				62.0	
85.12	6	180.9	153.3	77.3	507.2
86.00				111.4	
87.00				47.5	
87.81	6	66.3	164.8	30.5	432.1
89.00				134.7	
90.12	6.5	217.2	94.1	64.6	558.8
91.00				122.0	
92.12	6.5	213.7	181.8	60.3	552.7
93.00				107.1	
94.00				60.3	
94.78	6	140.1	173.5	7.1	493.5

Table C-14. Continued.

Train 4 – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
96.00				60.3	
97.14	6	224	149.2	204.9	569.3
98.00				58.2	
99.15	6	242.9	138.6	81.6	572.7
100.00				164.5	
101.00				102.8	
101.78	6.5	245.7	182.2	62.0	588.5
103.00				105.0	
104.13	6	323.2	181.3	62.0	667.9
105.00				124.1	
106.14	6	291.5	144.5	58.2	642.7
107.00				132.6	
108.00				45.4	
108.80	6.1	293.2	180.1	7.1	643.5
110.00				124.1	
111.12	6	260.7	186.3	77.3	609.9
112.00				117.7	
113.14	6	245.5	175.8	32.7	583.7
114.00				117.7	
115.00				77.3	
115.81	6.1	222.4	201.8	9.3	574.4
117.00				113.5	
118.13	6	303.2	171.3	22.0	634.2
119.00				79.4	
120.16	6	235.3	187.7	17.8	581.9
121.00				100.7	
122.00				62.4	
122.81	6.3	285.6	190.1	15.6	631.0
124.00				111.4	
125.13	6.1	204.2	143.3	58.2	527.3
126.00				115.6	
127.15	6	305	169.2	56.0	653.7
128.00				15.6	
129.00				96.5	
129.79	6	258.7	140.5	26.3	602.7
131.00				119.9	

Table C-14. Continued.

Train 4 – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
132.10	6	266.3	148.1	81.6	594.5
133.00				134.7	
134.09	6	312.1	138	68.8	655.1
135.00				194.3	
136.00				117.7	
136.78	6	293.6	130.9	70.9	642.6
138.00				119.9	
139.14	6	290.1	143.2	173.0	634.7
140.00				207.1	
141.09	6	356.2	145.5	73.1	696.5
142.00				160.3	
143.00				66.7	
143.82	6	272.6	213.5	45.4	644.1
145.00				251.7	
146.08	6	325.1	170.5	92.2	655.5
147.00				202.8	
148.09	6.5	288.7	169.9	83.7	619.6
149.00				179.4	
150.00				77.3	
150.81	6	290.1	195.8	28.4	627.1
152.00				132.6	
153.07	6	250.3	176.4	77.3	586.3
154.00				192.2	
155.10	6	291.4	185.8	53.9	615.6
156.00				96.5	
157.00				39.0	
157.78	6	292.4	187.4	19.9	625.6
159.00				5.0	
160.13	6	306.1	190.2	58.2	644.1
161.00				105.0	
162.10	6	268.9	168.8	49.7	608.8

Table C-15. Transfer data for Train 4 Fermentor 3. Red indicates data was estimated or interpolated based on other data.

Train 4 – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
0.00	5.5	194.5	94.1		450.7
1.82	5.5	209.8	78.6	53.9	480.4
3.98	6.5	193.2	157.3	81.6	479.2
6.03	6	174.9	184.4	92.2	485.3
8.07	6	186.7	168.5	60.3	471.0
10.87	6	122.1	155.6	122.0	429.7
12.98	5.5	193.1	179.1	251.7	509.0
15.06	6	43.8	182.9	143.3	393.1
17.83	6	149.7	143.7	15.6	447.5
19.34	6	122.6	210.8	136.9	447.4
22.05	6.5	117.4	195.3	156.0	452.9
24.83	6	125.5	119	145.4	461.3
26.34	6.5	130.2	156.6	179.4	454.4
29.07	6.5	72	158.2	150.0	408.8
31.74	6	200	138	349.6	516.8
34.15	6	228.3	134.5	11.4	579.5
36.27	0	0	221.5	100.7	272.9
38.79	6.5	55.6	134.8	105.0	371.6
40.00				217.7	
40.92	6	170.8	84.4	150.0	538.3
42.00				115.6	
42.34	6.5	202.1	105.6	130.5	550.7
44.00				151.8	
45.00				13.5	
45.80	6	210.9	212.2	132.6	551.8
47.00				268.7	
48.07	6	213.8	223.7	200.7	575.7
49.00				279.4	
50.00	6.5	204.8	207.4	107.1	571.3
51.00				156.0	
52.00				92.2	
52.96	6	224.3	210.4	19.9	570.3
54.00				153.9	
55.00				105.0	
56.00				70.9	

Table C-15. Continued.

Train 4 – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
57.11	6	208.4	188.9	92.2	542.6
58.00				130.5	
59.00				30.5	
59.79	6.5	285	190.7	105.0	628.6
61.00				168.8	
62.02	6.5	222.4	211.5	75.2	552.8
64.89	6.5	269.4	189	181.5	598.3
66.00				194.3	
66.75	6	277.3	175.2	62.4	589.7
68.00				230.5	
68.76	6	275.9	162.2	113.5	592.3
71.00				260.2	
71.83	6.5	270.9	173.1	109.2	595.9
73.00				153.9	
73.82	6.5	249.2	224.5	66.7	576.9
75.00				115.6	
76.07	6.5	202.4	178.4	51.8	513.5
77.00				132.6	
78.13	6	227.8	155.1	109.2	538.0
79.00				179.4	
80.00				88.0	
80.79	6	225.5	219.7	88.0	553.6
82.00				230.5	
83.08	6.5	212.5	160.9	170.9	546.6
84.00				150.0	
85.12	6	137.8	179.6	128.4	443.7
86.00				560.1	
87.00				130.5	
87.81	6	262.1	230.6	62.4	610.9
89.00				183.7	
90.12	6	273.8	135.7	117.7	619.9
91.00				247.5	
92.12	6	201.9	220.8	77.3	527.5
93.00				173.0	
94.00				85.8	
94.78	6	298.9	226.9	19.9	667.0

Table C-15. Continued.

Train 4 – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
96.00				187.9	
97.14	6	284.6	194.5	219.8	624.9
98.00				117.7	
99.15	6	297.6	168.4	132.6	613.8
100.00				400.6	
101.00				283.6	
101.78	6.5	277.4	224.4	98.6	608.6
103.00				164.5	
104.13	6	347	226	150.0	682.4
105.00				194.3	
106.14	6	345.6	195.7	102.8	681.8
107.00				253.9	
108.00				94.3	
108.80	6.1	319.1	230.4	73.1	652.7
110.00				202.8	
111.12	6	301.3	235.5	62.4	653.5
112.00				243.2	
113.14	6	278	214	1021.7	613.6
114.00				185.8	
115.00				66.7	
115.81	6.1	345.4	253.8	34.8	674.2
117.00				190.0	
118.13	6	288.1	202.3	96.5	640.3
119.00				175.2	
120.16	6	333.2	234.3	88.0	649.7
121.00				192.2	
122.00				96.5	
122.81	6.1	234.7	235.5	36.9	584.2
124.00				179.4	
125.13	6.1	360	166.4	128.4	692.3
126.00				185.8	
127.15	6	317.1	217.9	92.2	649.4
128.00				126.2	
129.00				94.3	
129.79	6	303.5	184.5	43.3	644.6
131.00				228.3	

Table C-15. Continued.

Train 4 – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
132.10	6	358.3	176.3	151.8	672.7
133.00				377.2	
134.09	6	350.5	181	419.8	678.5
135.00				260.2	
136.00				209.2	
136.78	6	346.8	179.9	92.2	655.9
138.00				287.9	
139.14	6	427.9	187.8	256.0	696.4
140.00				251.7	
141.09	6	351.3	185.8	198.6	671.7
142.00				219.8	
143.00				153.9	
143.82	6	365.5	285	105.0	673.1
145.00				390.0	
146.08	6	322.7	200.9	166.7	659.0
147.00				319.8	
148.09	6	334.2	200.8	136.9	640.7
149.00				324.1	
150.00				145.4	
150.81	6	289.7	232.8	68.8	612.2
152.00				202.8	
153.07	6	320.4	212.4	168.8	627.2
154.00				275.1	
155.10	6	329.9	210	145.4	635.3
156.00				209.2	
157.00				130.5	
157.78	6	353.4	220.6	94.3	657.9
159.00				5.0	
160.13	6	339.1	228.2	147.5	649.4
161.00				277.3	
162.10	6	326	208.7	151.8	639.7

Table C-16. Transfer data for Train 4 Fermentor 4. Red indicates data was estimated or interpolated based on other data.

Train 4 – Fermentor 4									
Day	**pH**	**Liquid removed**	**Cake added**	**Cake removed**	**Liquid added**	**Gas**	**Total ferm.**	***M* (cake removed)**	***I* (cake removed)**
		g	g	g	mL	cm^3	g	0	0
0.00	5.5	187.8	50.3	42.3	175		450.8	0.840	
1.82	6	185.9	49.2	76.2	175	117.7	484.0	0.840	
3.98	6.5	166.1	172.2	151.2	175	83.7	445.1	0.840	
6.03	6.5	133.8	194.8	164.3	175	542.0	379.3	0.840	
8.07	6.5	190.4	132.8	133	175	11.4	466.6	0.840	
10.87	5.5	214.2	163.2	142	175	1289.7	469.0	0.840	
12.98	6	98.1	195	198.2	175	102.8	377.3	0.840	
15.06	6	154.8	232.2	273.2	175	472.9	471.8	0.840	
17.83	5.5	155.1	145.1	178.4	175	662.2	464.4	0.840	
19.34	6	158.8	235.6	230.3	175	581.4	470.2	0.840	
22.05	6	162.5	230.8	268.7	175	492.1	509.9	0.840	
24.83	6	157	154.8	221	175	428.3	499.2	0.840	
26.34	6.5	118.1	180.8	255	300	711.2	468.3	0.840	
29.07	6	219.8	195	272.4	300	573.0	573.2	0.840	
31.74	6	282.7	154.8	192.2	300	624.0	596.1	0.840	
34.15	0	0	185.7	92.1	300	573.0	182.2	0.840	
36.27	6.5	76	194.4	195.9	300	64.6	353.5	0.840	
38.79	6	245.7	150.8	235.5	300	751.6	606.4	0.840	
40.00						226.2		0.840	
40.92	6	252.9	151.9	217.5	300	573.0	594.5	0.840	
42.00						228.3		0.840	
42.34	6.5	257.6	154.2	213.4	300	698.4	592.8	0.840	
44.00						345.3		0.840	
45.00						919.6		0.840	
45.80	6	285.7	253.1	226.6	300	326.2	591.2	0.840	
47.00						251.7		0.840	
48.07	6	279.1	285.6	286	300	738.8	645.5	0.840	
49.00						11.4		0.840	
50.00	6.5	275.2	273.9	314.8	300	347.4	669.4	0.840	
51.00						183.7		0.840	
52.00						677.1			
52.96	6	249.2	256.4	340.4	300	568.7	669.0	0.852	0.242
54.00						151.8			
55.00						598.4			

Table C-16. Continued.

Train 4 – Fermentor 4									
Day	**pH**	**Liquid removed**	**Cake added**	**Cake removed**	**Liquid added**	**Gas**	**Total ferm.**	***M* (cake removed)**	***I* (cake removed)**
		g	g	g	mL	cm^3	g	0	0
56.00						828.1			
57.11	6	334.1	223.1	233.7	300	624.0	647.5	0.810	0.294
58.00						130.5			
59.00						36.9			
59.79	6	257.9	234.3	276.8	300	738.8	618.8	0.838	0.266
61.00						249.6			
62.02	6	302.8	241.9	245.1	300	694.1	633.1	0.835	0.278
64.89	6	292.8	217.9	292.2	300	796.2	643.1	0.843	0.267
66.00						328.3			
66.75	5.5	300.7	187.6	246.1	300	596.3	635.2	0.865	0.267
68.00						641.0			
68.76	6	301.9	178.6	189.5	300	909.0	588.8	0.830	0.304
71.00						573.0			
71.83	6.5	282.3	198.1	59.4	300	1566.2	419.6	0.832	0.270
73.00						143.3			
73.82	6	218.8	249.9	255.6	300	149.6	548.5	0.834	0.262
75.00						345.3			
76.07	6.5	241.2	189.5	281.7	300	415.5	609.4	0.843	0.251
77.00						187.9			
78.13	6	259.9	165.3	164.1	300	800.5	534.7	0.824	0.285
79.00						430.4			
80.00						585.7			
80.79	6	256.2	247.8	153.9	300	475.1	509.7	0.810	0.302
82.00						1000.4			
83.08	6	149.3	195	245	300	777.1	475.3	0.831	0.250
84.00						573.0			
85.12	6	318.6	185.5	174.6	300	653.7	583.7	0.824	0.267
86.00						170.9			
87.00						894.1			
87.81	6	322.8	270.6	179.9	300	483.6	577.5	0.822	0.243
89.00						262.4			
90.12	6	229.3	181.8	241.4	300	324.1	564.9	0.837	0.270
91.00						832.4			
92.12	6	375.5	246.4	139.8	300	779.2	595.5	0.802	0.342
93.00						613.3			
94.00						815.4			

Table C-16. Continued.

Train 4 – Fermentor 4									
Day	**pH**	**Liquid removed**	**Cake added**	**Cake removed**	**Liquid added**	**Gas**	**Total ferm.**	***M* (cake removed)**	***I* (cake removed)**
		g	g	g	mL	cm^3	g	0	0
94.78	6	334.2	288.4	271.2	300	424.0	642.3	0.845	0.304
96.00						351.7			
97.14	6	321.7	234.8	276	300	1062.1	676.4	0.828	0.450
98.00						266.6			
99.15	6	316	184.6	222.1	300	796.2	629.5	0.838	0.492
100.0						624.0			
101.0						1028.1			
101.7	6.5	386	250.3	126.1	300	481.4	592.5	0.789	0.348
103.0						1157.8			
104.1	6	388.8	257.9	173.7	300	573.0	640.7	0.802	0.348
105.0						309.2			
106.1	6	360.6	232.2	214.7	300	1004.7	652.1	0.825	0.331
107.0						287.9			
108.0						977.0			
108.8	6.1	353	255.4	202.5	300	621.8	629.1	0.837	0.346
110.0						247.5			
111.1	6	317	273.3	263.1	300	906.8	655.3	0.863	0.315
112.0						217.7			
113.1	6	379	250.1	211.5	300	585.7	668.5	0.822	0.324
114.0						256.0			
115.0						672.9			
115.8	6.1	317.6	250.6	244	300	602.7	642.2	0.857	0.304
117.0						624.0			
118.1	6	359.5	254.5	207.2	300	981.3	644.6	0.845	0.354
119.0						313.4			
120.1	6	283.8	238.2	281.9	300	1145.1	645.4	0.864	0.366
121.0						479.3			
122.0						1177.0			
122.8	6.1	385.2	271.1	170.9	300	347.4	636.3	0.830	0.373
124.0						466.6			
125.1	6.1	360.3	198.7	228.1	300	1168.5	665.7	0.829	0.264
126.0						509.1			
127.1	6	346.5	250.2	164.9	300	1172.7	588.4	0.825	0.360
128.0						460.2			
129.0						1125.9			
129.7	6	380.1	225.6	184.6	300	434.7	643.2	0.838	0.379

Table C-16. Continued.

Train 4 – Fermentor 4									
Day	**pH**	**Liquid removed**	**Cake added**	**Cake removed**	**Liquid added**	**Gas**	**Total ferm.**	***M* (cake removed)**	***I* (cake removed)**
		g	g	g	mL	cm^3	g	0	0
131.0						426.1			
132.1	6	364	190.7	165	300	1170.6	614.3	0.828	0.326
133.0						581.4			
134.0	6	346.6	209	142.9	300	1157.8	571.7	0.811	0.366
135.0						1091.9			
136.0						1098.3			
136.7	6	410.6	189	107.5	300	562.3	605.1	0.712	0.482
138.0						498.5			
139.1	6	378.9	156.3	87.4	300	1019.6	586.0	0.777	0.384
140.0						966.4			
141.0	6	348.6	206.2	127	300	845.2	554.2	0.744	0.266
142.0						285.8			
143.0						641.0			
143.8	6	305.5	229.2	213.6	300	366.6	598.8	0.843	0.225
145.0						468.7			
146.0	6	349.9	237.2	200.7	300	866.4	624.6	0.819	0.324
147.0						328.3			
148.0	6	321.1	207.3	233.6	300	1140.8	632.7	0.805	0.359
149.0						264.5			
150.0						1057.9			
150.8	6	330.4	244.9	187.4	300	613.3	597.6	0.853	0.308
152.0						326.2			
153.0	6	344.3	219.2	212.7	300	1432.2	639.0	0.851	0.316
154.0						613.3			
155.1	6	369.5	215.4	160	300	1200.4	613.1	0.827	0.290
156.0						685.6			
157.0						889.8			
157.7	6	359.1	225.1	176.4	300	470.8	610.9	0.840	0.353
159.0						5.0			
160.1	6	361.6	233	178.1	300	1423.7	618.4	0.821	0.305
161.0						130.5			
162.1	6	281.4	222.4	224.5	300	175.2	580.0	0.862	0.356

Table C-17. Transfer data for Train P Fermentor 1. Red indicates data was estimated or interpolated based on other data.

Train P – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
0.00	6.5	79.4	35		448.7	0.960	0.365
1.76	6	74.1	35	119.9	447.6	0.960	0.365
3.90	6	104.4	35	277.3	512.2	0.960	0.365
5.97	6.5	43.8	35	58.2	445.5	0.960	0.365
8.02	6.5	81.2	35	253.9	474.7	0.960	0.365
10.79	7	44.8	35	11.4	443.4	0.960	0.365
12.91	6.5	43.9	35	245.4	439.5	0.960	0.365
15.00	6	16	35	347.4	416.1	0.960	0.365
17.78	6	30.5	35	277.3	421.9	0.960	0.365
19.99	6.5	5.5	35	73.1	376.4	0.960	0.365
22.01	6.5	10	35	32.7	292.4	0.960	0.365
24.77	6	1	35	53.9	231.0	0.960	0.365
26.94		0	35	79.4	233.9	0.960	0.365
28.26		0	35	13.5	274.1	0.960	0.365
31.70		0	35	51.8	268.9	0.960	0.365
34.11		0	35	49.7	247.5	0.960	0.365
36.21		0	35	88.0	277.8	0.960	0.365
38.74		0	35	36.0	323.7	0.960	0.365
40.00				24.1			
40.87	7	21.6	35	200.0	332.2	0.960	0.365
42.00				17.8			
43.09	6.5	54.5	35	39.0	360.0	0.960	0.365
44.00				19.9			
45.00				60.3			
45.74	6.5	56.2	35	51.8	369.9	0.960	0.365
47.00				26.3			
48.00	6.5	46.3	35	73.1	390.5	0.960	0.365
49.00				187.9			
50.10	6.5	17.8	35	79.4	373.2	0.960	0.365
51.00				277.3			
52.00				66.7			
52.91	6.5	96.8	35	28.4	407.2	0.960	0.365
54.00				277.3			
55.00				126.2			

Table C-17. Continued.

Train P – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
56.00				113.5			
57.04	6.5	120.2	35	187.9	439.0	0.960	0.365
58.00				294.3			
59.00				36.9			
59.73	6	85.1	35	64.6	414.8	0.960	0.365
61.00				381.5			
61.96	6	169.3	35	200.0	479.3	0.960	0.365
64.85	6.5	177.8	35	107.1	491.1	0.960	0.365
66.00				258.1			
66.69	6.5	132	35	92.2	455.9	0.960	0.365
68.00				262.4			
68.69	6.5	118.6	35	141.1	437.2	0.960	0.365
71.00				402.7			
71.76	6	195.3	35	136.9	509.7	0.960	0.365
73.00				347.4			
73.75	6.5	188.1	35	30.5	523.5	0.960	0.365
75.00				370.8			
76.01	6.5	173.1	35	102.8	478.2	0.960	0.365
77.00				292.1			
78.07	6	126.3	35	183.7	434.5	0.960	0.365
79.00				409.1			
80.00				88.0			
80.73	6.5	129.7	35	77.3	456.9	0.960	0.365
82.00				298.5			
83.02	6.5	126.6	35	156.0	469.2	0.966	0.359
84.00				200.0			
85.06	6	107.1	35	158.1	447.8	0.963	0.342
86.00				360.2			
87.00				160.3			
87.73	6	144.5	35	77.3	469.9	0.969	0.394
89.00				264.5			
90.04	6.5	145.7	35	122.0	484.7	0.957	0.464
91.00				270.9			
92.08	6	131.8	35	160.3	469.1	0.967	0.687
93.00				317.7			
94.00				177.3			

Table C-17. Continued.

Train P – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
94.72	6.5	178.3	35	30.5	489.6	0.971	0.926
96.00				313.4			
97.09	6	133.9	35	279.4	466.0	0.965	0.800
98.00				294.3			
99.07	6.5	108.1	35	153.9	436.4	0.967	0.632
100.0				287.9			
101.0				192.2			
101.7	6	167.1	35	128.4	479.9	0.960	0.466
103.0				390.0			
104.0	6	108.3	35	200.0	430.3	0.962	0.523
105.0				266.6			
106.0	6	182.3	35	122.0	511.2	0.964	0.515
107.0				253.9			
108.0				109.2			
108.7	6.1	163.0	35	7.1	497.5	0.967	-0.037
110.0				311.3			
111.0	6	194.8	35	115.6	515.7	0.964	0.628
112.0				196.4			
113.0	6	191	35	187.9	510.1	0.962	0.634
114.0				228.3			
115.0				119.9			
115.7	6.5	157.3	35	105.0	520.8	0.969	0.605
117.0				349.6			
118.0	6	125.2	35	90.1	455.6	0.964	0.698
119.0				319.8			
120.0	6	131.9	35	134.7	444.8	0.965	0.677
121.0				260.2			
122.0				128.4			
122.7	6.1	129	35	32.7	483.8	0.960	0.355
124.0				251.7			
125.0	6	141.3	35	168.8	505.1	0.968	0.572
126.0				279.4			
127.0	6	110.3	35	175.2	431.2	0.968	0.556
128.0				296.4			
129.0				164.5			
129.7	6	122.5	35	49.7	492.2	0.961	0.777

Table C-17. Continued.

Train P – Fermentor 1							
Day	**pH**	**Liquid removed**	**Feedstock added**	**Gas**	**Total ferm.**	***M* (liq. Removed)**	***I* (liq. Removed)**
		g	g	cm^3	g	0	0
131.0				290.0			
132.0	6	164.3	35	151.8	529.1	0.966	0.310
133.0				334.7			
134.0	6	123.4	35	168.8	458.2	0.964	1.054
135.0				387.9			
136.0				88.0			
136.7	6.5	125.9	35	7.1	462.9	0.960	0.550
138.0				455.9			
139.0	6	124.8	35	185.8	451.3	0.963	0.911
140.0				351.7			
141.0	6	112.2	35	102.8	430.2	0.964	0.998
142.0				328.3			
143.0				149.6			
143.7	6.5	94.4	35	7.1	409.0	0.962	0.692
145.0				219.8			
146.0	6.5	146.8	35	132.6	499.7	0.960	0.486
147.0				245.4			
148.0	6.5	150.3	35	105.0	494.7	0.965	0.417
149.0				283.6			
150.0				185.8			
150.7	6.5	182.3	35	107.1	558.0	0.966	0.653
152.0				317.7			
153.0	6	160.7	35	170.9	516.1	0.964	0.610
154.0				328.3			
155.0	6	152	35	217.7	497.6	0.968	0.520
156.0				364.5			
157.0				170.9			
157.7	6.5	135.4	35	26.3	480.1	0.963	0.841
159.0				5.0			
160.0	6	122.4	35	170.9	475.9	0.967	0.522
161.0				379.4			
162.0	6	186.3	35	117.7	540.7	0.970	0.908

Table C-18. Transfer data for Train P Fermentor 2. Red indicates data was estimated or interpolated based on other data.

Train P – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
0.00	5.5	163.2	123.2		448.7
1.76	5.5	216.3	115.7	43.3	476.7
3.90	6	181.7	150	98.6	504.4
5.97	6	150.5	162.7	167.0	475.8
8.02	5.5	150.3	134.5	85.8	505.3
10.79	6	144.2	139.6	79.4	481.6
12.91	6.5	122.4	136.6	117.7	465.7
15.00	6	132.5	141.1	124.1	470.5
17.78	6	85.1	132.4	230.5	407.5
19.99	6.5	89	211.9	192.2	436.5
22.01	6.5	51.9	123.4	162.4	464.5
24.77	6.5	36.4	71	181.5	360.4
26.94	6.5	80	74.9	264.5	430.4
28.26	6.5	72.1	115.1	167.0	453.1
31.70	6.5	48.4	109.9	398.5	418.9
34.11	6	80.7	88.5	79.4	448.3
36.21	6	126.6	118.8	300.7	499.1
38.74	6	134.2	164.7	277.3	492.0
40.00				132.6	
40.87	6.5	166.3	151.6	167.0	521.6
42.00				85.8	
43.09	6	169.4	146.5	113.5	515.0
44.00				168.8	
45.00				153.9	
45.74	6	196.6	154.7	122.0	546.6
47.00				168.8	
48.00	6.5	181.1	185.2	183.7	539.3
49.00				181.5	
50.10	6	210.9	196.4	198.6	554.0
51.00				181.5	
52.00				170.9	
52.91	6.5	251.3	151.4	79.4	605.2
54.00				298.5	
55.00				260.2	
56.00				207.1	

Table C-18. Continued.

Train P – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
57.04	6.5	216.3	159.8	247.5	548.8
58.00				245.4	
59.00				36.9	
59.73	6	287.9	170.7	130.5	636.1
61.00				204.9	
61.96	6	293.1	151	124.1	649.3
64.85	6.5	249.5	154.3	119.9	615.5
66.00				111.4	
66.69	6.5	245.3	164.9	88.0	555.7
68.00				124.1	
68.69	6	312.5	159.6	60.3	657.1
71.00				226.2	
71.76	6	331.6	155.4	90.1	673.2
73.00				290.0	
73.75	6	285.6	176.4	64.6	640.4
75.00				204.9	
76.01	6	238.8	146.1	113.5	590.0
77.00				136.9	
78.07	6	270.8	149.2	185.8	600.8
79.00				253.9	
80.00				168.8	
80.73	6	274.6	168.2	190.0	643.8
82.00				177.3	
83.02	6.5	253.9	183.6	198.6	604.4
84.00				167.0	
85.06	6	272.7	181.7	158.1	617.4
86.00				190.0	
87.00				141.1	
87.73	6	293.2	166.4	58.2	629.9
89.00				236.8	
90.04	6	270.7	180	134.7	615.1
91.00				194.3	
92.08	6	297.4	178.3	160.3	650.9
93.00				130.5	
94.00				128.4	
94.72	6	281.8	152.3	49.7	621.9

Table C-18. Continued.

Train P – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
96.00				247.5	
97.09	6	239.4	173.1	268.7	602.2
98.00				224.1	
99.07	6	287	169.3	102.8	638.2
100.00				145.4	
101.00				113.5	
101.73	6	238.2	153.8	90.1	573.4
103.00				249.6	
104.08	6	316.7	163	167.0	661.5
105.00				230.5	
106.06	6	302.1	169.9	207.1	636.0
107.00				194.3	
108.00				202.8	
108.73	6.1	320.7	175.5	70.9	672.8
110.00				224.1	
111.02	6	313.7	161.9	156.0	660.3
112.00				162.4	
113.06	6	326.3	160.5	185.8	648.4
114.00				224.1	
115.00				92.2	
115.74	6.1	264	204.5	119.9	621.9
117.00				258.1	
118.06	6	247.5	171.4	109.2	591.7
119.00				243.2	
120.06	6	290.7	153.9	143.3	635.3
121.00				173.0	
122.00				113.5	
122.76	6.1	316.8	195.8	56.0	666.0
124.00				217.7	
125.06	6	236.7	204.8	115.6	579.2
126.00				181.5	
127.09	6	301.5	161.9	88.0	636.0
128.00				164.5	
129.00				66.7	
129.73	6	335.9	210.7	60.3	680.5
131.00				147.5	

Table C-18. Continued.

Train P – Fermentor 2					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
132.05	6	265.6	205.8	70.9	599.4
133.00				126.2	
134.04	6	262.2	175.8	77.3	591.9
135.00				126.2	
136.00				113.5	
136.74	6	264	178	19.9	609.6
138.00				215.6	
139.09	6	236.6	167.5	200.7	553.3
140.00				173.0	
141.04	6	211.9	159	122.0	550.9
142.00				194.3	
143.00				109.2	
143.70	6	313.1	155.6	53.9	658.8
145.00				153.9	
146.01	6	305.9	193.9	81.6	650.4
147.00				141.1	
148.02	6	363.7	185.4	81.6	691.6
149.00				143.3	
150.00				66.7	
150.73	6	325.8	216.7	77.3	678.4
152.00				130.5	
153.01	6	300.9	196.4	62.4	652.1
154.00				183.7	
155.03	6	290.9	186.6	64.6	630.1
156.00				196.4	
157.00				66.7	
157.72	6	284.1	185.7	98.6	634.4
159.00				5.0	
160.05	6	375.4	194.5	309.2	702.6
161.00				196.4	
162.04	6	221.2	195.4	198.6	571.2

Table C-19. Transfer data for Train P Fermentor 3. Red indicates data was estimated or interpolated based on other data.

Train P – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
0.00	5.5	182.8	115.9		449.5
1.76	6	210	83.3	36.9	471.6
3.90	6	214.2	179.9	90.1	498.3
5.97	6	189.4	174	68.8	483.4
8.02	6	184.2	195.5	88.0	462.6
10.79	6	168.6	183	68.8	475.3
12.91	6	176.2	185.9	64.6	473.7
15.00	6.5	109.3	185.1	47.5	449.1
17.78	6	142	160.8	234.7	445.6
19.99	6	105.8	265.2	233.0	455.9
22.01	6	120.8	242	324.1	492.3
24.77	6	134.1	101	179.4	487.9
26.94	6	157.4	125.1	226.2	487.2
28.26	6.5	125.2	202.1	233.0	442.9
31.70	6	208.6	186.4	402.7	544.5
34.11	6	202.6	162.1	233.0	580.3
36.21	6	200.9	197.3	464.4	554.1
38.74	6	237.4	228.5	292.1	587.3
40.00				173.0	
40.87	6.5	220.2	212.9	233.0	602.4
42.00				119.9	
43.09	6	256.2	198.1	200.7	568.4
44.00				170.9	
45.00				219.8	
45.74	6	242.7	210.7	194.3	584.5
47.00				285.8	
48.00	6	263.4	249.4	298.5	597.7
49.00				204.9	
50.10	6	310.5	245.5	270.9	669.6
51.00				211.3	
52.00				236.8	
52.91	6	258.3	211.3	187.9	629.5
54.00				298.5	
55.00				407.0	
56.00				417.6	

Table C-19. Continued.

Train P – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
57.04	6	344.2	198.3	377.2	596.7
58.00				211.3	
59.00				17.8	
59.73	6	378	225.4	117.7	690.2
61.00				179.4	
61.96	6	319.8	213.2	94.3	621.8
64.85	6.5	309	226.3	115.6	617.7
66.00				136.9	
66.69	6	364.5	181.3	94.3	652.1
68.00				130.5	
68.69	6	370.5	210.2	94.3	638.5
71.00				441.0	
71.76	6	342.3	203	300.7	599.2
73.00				541.0	
73.75	6	300.3	237.2	213.4	587.7
75.00				202.8	
76.01	6	306.9	203.3	239.0	613.0
77.00				170.9	
78.07	6	348.1	185.2	236.8	596.6
79.00				107.1	
80.00				436.8	
80.73	6	315.2	243.4	264.5	624.6
82.00				179.4	
83.02	6	324.5	240.1	304.9	604.5
84.00				233.0	
85.06	6	336.1	232.4	160.3	639.3
86.00				153.9	
87.00				198.6	
87.73	6	324.6	209.1	224.1	591.1
89.00				228.3	
90.04	6	356.2	230.4	385.7	625.9
91.00				192.2	
92.08	6	330.3	237.8	328.3	625.3
93.00				215.6	
94.00				219.8	
94.72	6	312.6	198.4	177.3	625.8

Table C-19. Continued.

Train P – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
96.00				436.8	
97.09	6	342.3	241.9	387.9	640.9
98.00				134.7	
99.07	6.5	281.1	226.5	304.9	586.8
100.00				175.2	
101.00				266.6	
101.73	6	371	195	262.4	655.5
103.00				302.8	
104.08	6	339.6	213.8	233.0	635.2
105.00				232.6	
106.06	6	379.4	209.8	364.5	616.3
107.00				224.1	
108.00				353.8	
108.73	6.1	363.9	233.6	175.2	635.9
110.00				264.5	
111.02	6	354.7	214.5	304.9	644.2
112.00				115.6	
113.06	6	328.2	188.6	373.0	629.1
114.00				253.9	
115.00				381.5	
115.74	6.1	301.4	268.4	217.7	604.9
117.00				294.3	
118.06	6	339.1	221.6	258.1	647.0
119.00				222.0	
120.06	6	373.6	204.5	296.4	653.4
121.00				187.9	
122.00				336.8	
122.76	6.1	287.7	251	151.8	599.4
124.00				200.7	
125.06	6	340	253.3	190.0	650.8
126.00				219.8	
127.09	6	387.4	202.4	107.1	675.3
128.00				190.0	
129.00				92.2	
129.73	6	306.4	261.3	68.8	613.6
131.00				158.1	

Table C-19. Continued.

Train P – Fermentor 3					
Day	**pH**	**Liquid removed**	**Cake added**	**Gas**	**Total ferm.**
		g	g	cm^3	g
132.05	6	306.1	245.6	107.1	611.3
133.00				128.4	
134.04	6	315.4	211.5	83.7	642.5
135.00				126.2	
136.00				75.2	
136.74	6	267.1	229.6	141.1	575.8
138.00				170.9	
139.09	6	256.9	190.2	232.6	545.4
140.00				236.8	
141.04	6	361.3	204	247.5	660.8
142.00				243.2	
143.00				134.7	
143.70	6	355	207.3	70.9	623.5
145.00				192.2	
146.01	6	397.2	244.4	85.8	661.5
147.00				143.3	
148.02	6	380	219.3	113.5	634.5
149.00				147.5	
150.00				73.1	
150.73	6	361.4	275.3	45.4	649.3
152.00				224.1	
153.01	6	335.1	253.6	136.9	656.6
154.00				187.9	0.0
155.03	6	339.6	231.8	192.2	632.3
156.00				202.8	
157.00				105.0	
157.72	6	355	242	183.7	659.7
159.00				5.0	
160.05	6	298.9	227.7	185.8	593.3
161.00				390.0	0.0
162.04	6	404.1	251.4	285.8	639.2

Table C-20. Transfer data for Train P Fermentor 4. Red indicates data was estimated or interpolated based on other data.

Train P – Fermentor 4									
Day	**pH**	**Liquid removed**	**Cake added**	**Cake removed**	**Liquid added**	**Gas**	**Total ferm.**	***M* (cake removed)**	***I* (cake removed)**
		g	g	g	mL	cm^3	g	0	0
0.00	5.5	180	89.8	65.9	175		448.9	0.820	
1.76	5.5	204.6	52.1	21.5	175	68.8	466.8	0.820	
3.90	6	172.4	301.4	180.6	175	58.2	473.8	0.820	
5.97	6	179.4	174	166.6	175	73.1	466.0	0.820	
8.02	6	184.6	179.9	172.9	175	11.4	471.6	0.820	
10.79	6	180.6	195.7	183.7	175	66.7	462.6	0.820	
12.91	6	170.8	189.4	198.4	175	85.8	473.8	0.820	
15.00	6	148.5	230.9	259.2	175	249.6	470.8	0.820	
17.78	6	165.8	170.4	196.2	175	715.4	485.6	0.820	
19.99	6	141.6	275.2	306.7	175	604.8	467.1	0.820	
22.01	6	176	294.6	288.1	175	256.0	538.4	0.820	
24.77	6	184.5	154.8	218.8	175	426.1	542.5	0.820	
26.94	6	149.4	160.9	190.3	300	455.9	472.8	0.820	
28.26	6	243.1	225.8	257.4	300	564.4	586.4	0.820	
31.70	6	290.3	228.3	257.2	300	1128.	613.2	0.820	
34.11	6	254.3	245.8	300.3	300	1036.	602.8	0.820	
36.21	6	286.8	256.5	306.3	300	249.6	630.6	0.820	
38.74	6	297.8	272.7	271.7	300	394.2	643.8	0.820	
40.00						241.1		0.820	
40.87	6	276.6	301.1	290.4	300	371.0	650.9	0.820	
42.00						124.1		0.820	
43.09	6	290.1	216.3	273.5	300	555.9	641.3	0.820	
44.00						460.2		0.820	
45.00						758.0		0.820	
45.74	6	303.3	258.5	217.2	300	319.8	600.7	0.820	
47.00						536.8		0.820	
48.00	6	342.3	251	213.3	300	606.9	639.5	0.820	
49.00						560.1		0.820	
50.10	6	308	280.8	308.7	300	670.8	629.9	0.820	
51.00						292.1		0.820	
52.00						617.6		0.820	
52.91	6	306.8	288.5	272.6	300	264.5	658.6	0.839	0.267
54.00						617.6			
55.00						381.5			

Table C-20. Continued.

Train P – Fermentor 4									
Day	**pH**	**Liquid removed**	**Cake added**	**Cake removed**	**Liquid added**	**Gas**	**Total ferm.**	***M* (cake removed)**	***I* (cake removed)**
		g	g	g	mL	cm^3	g	0	0
56.00						213.4			
57.04	6	394.3	156.8	133.1	300	283.6	664.6	0.809	0.292
58.00						375.1			
59.00						36.9			
59.73	6	322.4	233.6	194.5	300	211.3	592.0	0.817	0.293
61.00						222.0			
61.96	6	325.2	221.2	201.4	300	181.5	612.5	0.814	0.277
64.85	6	307.4	231	221.4	300	379.4	606.4	0.833	0.240
66.00						85.8			
66.69	6	348.7	174.9	148.8	300	224.1	616.6	0.815	0.263
68.00						219.8			
68.69	6	307.2	184.2	179.5	300	353.8	596.5	0.810	0.267
71.00						936.6			
71.76	6	296.1	165.9	169.1	300	243.2	593.3	0.820	0.265
73.00						717.5			
73.75	6	303.1	209.4	210.2	300	219.8	597.9	0.834	0.262
75.00						460.2			
76.01	6	306.2	215.4	197.4	300	328.3	589.2	0.830	0.277
77.00						268.7			
78.07	6	335.1	139.7	109.7	300	579.3	599.1	0.825	0.247
79.00						598.4			
80.00						481.4			
80.73	6	281.9	227.8	225.8	300	268.7	593.5	0.825	0.286
82.00						424.0			
83.02	6	326.2	201.4	191.6	300	398.5	610.4	0.822	0.270
84.00						371.0			
85.06	6	284	223	225.3	300	464.4	589.3	0.825	0.275
86.00						375.1			
87.00						260.2			
87.73	6	338.9	181.6	147	300	258.1	598.3	0.806	0.225
89.00						594.2			
90.04	6	315.9	189.4	172.9	300	464.4	593.4	0.810	0.305
91.00						526.1			
92.08	6	312.4	220.1	209.5	300	370.8	595.8	0.833	0.298
93.00						615.4			
94.00						349.6			

Table C-20. Continued.

Train P – Fermentor 4									
Day	**pH**	**Liquid remove**	**Cake added**	**Cake remove**	**Liquid**	**Gas**	**Total ferm.**	***M* (cake removed**	***I* (cake removed**
		g	g	g	mL	cm^3	g	0	0
94.72	6	338.6	217.6	177.7	300	177.3	593.9	0.822	0.397
96.00						92.2			
97.09	6	269.1	218.8	226	300	513.4	570.3	0.820	0.500
98.00						168.8			
99.07	6	348.6	219	168.5	300	275.1	597.3	0.796	0.458
100.0						543.1			
101.0						362.3			
101.7	6	343.4	185.5	144.7	300	273.0	596.6	0.820	0.292
103.0						660.1			
104.0	6	325.5	215.4	188	300	371.0	592.1	0.817	0.313
105.0						483.6			
106.0	6	346.6	152.7	107.8	300	441.0	595.7	0.812	0.327
107.0						526.1			
108.0						451.7			
108.7	6.1	331.2	156.8	153.4	300	198.6	591.8	0.787	0.303
110.0						613.3			
111.0	6	339.2	208.9	169.5	300	285.8	593.8	0.789	0.324
112.0						266.6			
113.0	6	315.1	195.5	177.3	300	579.3	590.9	0.796	0.373
114.0						564.4			
115.0						273.0			
115.7	6.1	300.2	222.5	211.9	300	370.8	593.0	0.844	0.285
117.0						564.4			
118.0	6	355.8	229.4	166.4	300	481.4	603.4	0.814	0.297
119.0						492.1			
120.0	6	310.4	190.3	196.7	300	332.6	610.8	0.814	0.330
121.0						581.4			
122.0						407.0			
122.7	6.1	320.5	228.4	186.1	300	183.7	589.3	0.830	0.493
124.0						424.0			
125.0	6	346	233.8	188	300	324.1	608.6	0.861	0.154
126.0						511.2			
127.0	6	323.8	196.3	190.6	300	394.2	612.1	0.817	0.296
128.0						185.8			
129.0						415.5			
129.7	6	274.4	229.9	238.4	300	198.6	592.2	0.830	0.346

Table C-20. Continued.

Train P – Fermentor 4									
Day	**pH**	**Liquid removed**	**Cake added**	**Cake removed**	**Liquid added**	**Gas**	**Total ferm.**	***M* (cake removed)**	***I* (cake removed)**
		g	g	g	mL	cm^3	g	0	0
131.0						183.7			
132.0	6	323.8	227.9	207.4	300	368.7	612.8	0.837	0.318
133.0						94.3			
134.0	6	288.7	244.6	240	300	311.3	607.4	0.833	0.247
135.0						277.3			
136.0						434.7			
136.7	6	333.2	230.1	212.6	300	181.5	623.3	0.836	0.246
138.0						581.4			
139.0	6	371.4	184.7	120	300	396.4	600.7	0.827	0.318
140.0						328.3			
141.0	6	331.3	209.5	176.5	300	336.8	592.3	0.824	0.348
142.0						283.6			
143.0						332.6			
143.7	6	369.4	181.8	112.8	300	109.2	594.4	0.792	0.287
145.0						343.2			
146.0	6	315	186.1	167	300	321.9	589.9	0.803	0.404
147.0						149.6			
148.0	6	353.3	178.3	125.9	300	379.4	594.9	0.764	0.396
149.0						194.3			
150.0						417.6			
150.7	6	307	208.8	206.3	300	253.9	598.5	0.780	0.372
152.0						273.0			
153.0	6	301.2	243.8	213.2	300	441.0	593.2	0.817	0.334
154.0						-			
155.0	6	351.8	214.8	192.1	300	432.5	623.1	0.804	0.311
156.0						547.4			
157.0						270.9			
157.7	6	330.2	226.9	184.6	300	211.3	594.3	0.811	0.796
159.0						5.0			
160.0	6	366.3	218.2	163.5	300	247.5	607.0	0.819	0.253
161.0						385.7			
162.0	6	277.3	160.9	163.4	300	194.3	573.8	0.851	0.279

Table C-21. Acid and aceq concentrations (g/L) for Train 1. Red indicates missing data was interpolated or estimated.

	Train 1							
	F1		F2		F3		F4	
Day	Total	Aceq	Total	Aceq	Total	Aceq	Total	Aceq
0	25.14	37.19	12.10	18.73	9.52	14.67	10.09	15.49
2	20.06	30.02	16.21	24.19	11.44	17.21	5.02	7.67
4	19.90	29.77	16.07	23.56	10.34	15.01	4.85	6.92
6	20.84	29.92	16.29	23.58	10.97	15.53	5.59	7.65
8	22.43	32.05	16.83	23.80	11.45	15.96	6.01	8.20
11	22.42	31.30	17.49	24.40	12.07	16.65	6.52	8.80
13	23.35	31.56	17.08	23.41	12.71	17.28	6.51	8.70
15	21.07	28.63	17.75	23.85	13.32	17.88	7.18	9.55
18	27.31	37.64	20.47	27.69	14.50	19.28	7.34	9.61
20	25.35	34.07					7.00	9.24
22	28.06	37.97					7.00	9.24
25	28.60	38.03					7.00	9.24
41	26.87	36.19					8.00	10.56
46	26.58	35.06					9.00	11.88
48	25.36	33.49	23.56	31.26	19.36	25.58	10.44	13.80
50	27.37	35.89						12.14
53	26.07	34.26						12.14
57	24.56	33.04						12.14
60	26.50	35.24	21.49	29.09	14.40	19.53	7.76	10.48
62	24.77	33.47	20.33		13.70		7.85	10.40
65	24.52	32.66	20.33		13.70		7.85	10.40
67	24.00	32.24	19.18	25.80	13.00		7.94	10.44
69	23.31	30.45	19.54		12.75		7.58	10.00
72	22.87	30.07	19.54		12.75		7.58	10.00
74	23.47	31.10	19.89	26.18	12.50	16.78	7.22	9.29
76	19.65	26.33	19.29		12.25		6.62	8.50
78	19.86	26.39	19.29		12.25		6.62	8.50
81	21.90	29.41	18.68	25.10	12.00		6.01	8.07
83	20.32	27.68	18.02		11.87		5.54	7.44
85	20.10	26.93	18.02		11.87		5.77	7.88
88	20.58	27.78	17.37	23.53	11.73	15.64	5.99	8.32
90	20.79	27.90	16.80		11.64		5.80	7.68
92	18.98	25.20	16.80		11.64		5.47	7.24
95	19.70	25.76	16.23	21.21	11.54	15.29	5.72	7.50
97	22.42	28.92	16.79		11.75		5.67	7.45
99	22.99	29.93	16.79		11.75		5.95	7.70

Table C-21. Continued.

Train 1								
	F1		**F2**		**F3**		**F4**	
Day	**Total**	**Aceq**	**Total**	**Aceq**	**Total**	**Aceq**	**Total**	**Aceq**
102	21.66	28.25	17.34	22.46	11.96	15.46	6.18	8.22
104	21.76	28.26	17.60		11.05		7.20	9.32
106	21.44	28.01	17.60		11.05		4.45	5.78
109	19.39	25.46	17.86	23.13	10.13	13.22	6.80	8.87
111	24.82	32.12	17.36		11.25		4.98	6.42
113	23.27	30.36	17.36		11.25		7.08	9.22
116	25.55	33.73	16.86	22.07	12.36	16.07	5.76	7.57
118	21.93	28.76	16.83		11.75		5.76	7.46
120	20.21	26.63	16.83		11.75		5.69	7.44
123	22.32	30.33	16.79	22.10	11.13	14.63	5.39	7.04
125	21.46	28.89	16.39		10.99		5.19	6.76
127	20.97	28.73	16.39		10.99		5.79	7.63
130	21.10	29.00	15.99	21.41	10.85	14.40	5.36	7.07
132	20.80	27.76	15.66		10.93		5.47	7.19
134	19.86	26.97	15.66		10.93		5.71	7.57
137	18.94	26.50	15.33	20.57	11.02	14.63	5.46	7.27
139	20.93	28.20	15.53		10.93		5.77	7.60
141	18.30	25.29	15.53		10.93		5.03	6.59
144	19.91	27.38	15.73	21.25	10.85	14.45	5.63	7.48
146	19.03	25.98	15.26		11.03		5.11	6.73
148	20.28	27.46	15.26		11.03		5.36	7.10
151	21.75	29.38	14.79	19.72	11.21	14.84	5.41	7.06
153	20.80	27.79	16.03		11.59		5.70	7.56
155	19.34	25.86	16.03		11.59		5.66	7.60
158	21.25	28.37	17.26	23.05	11.98	16.11	6.26	8.45
160	21.57	28.64	16.83		10.13		5.30	7.07
162	21.65	28.85	16.41	21.96	8.28	11.23	5.11	6.83

Table C-22. Acid and aceq concentrations (g/L) for Train 2. Red indicates missing data was interpolated or estimated.

	Train 2							
	F1		F2		F3		F4	
Day	**Total**	**Aceq**	**Total**	**Aceq**	**Total**	**Aceq**	**Total**	**Aceq**
0	15.17	23.18	27.93	41.44	11.07	17.08	10.42	16.06
2	21.34	31.48	17.25	25.36	11.29	16.98	5.15	7.96
4	20.13	28.54	16.56	23.86	11.10	16.15	4.90	7.02
6	20.82	28.80	15.60	21.93	11.34	15.92	5.86	8.08
8	20.73	28.03	17.12	23.87	11.54	15.86	5.97	8.07
11	21.10	28.73	17.06	23.38	12.59	17.10	6.84	9.20
13	24.13	32.13	16.67	22.45	13.29	17.88	7.95	10.56
15	21.84	28.60	19.47	25.95	14.03	18.53	8.20	10.85
18	23.17	30.07	20.73	27.40	14.87	19.63	9.30	12.03
20	27.58	35.25					8.00	10.40
22	26.49	33.57					8.00	10.40
25	28.74	36.82					8.00	10.40
41	32.09	41.54					8.50	11.05
46	30.15	38.54					9.00	11.70
48	27.01	34.30	23.54	30.13	18.78	24.07	10.13	12.97
50	30.56	38.48						12.75
53	30.13	38.21						12.75
57	26.28	33.63						12.75
60	27.76	35.09	21.96	29.02	15.85	20.86	8.97	12.69
62	26.84	34.42	20.88		15.78		8.77	10.40
65	25.18	32.55	20.88		15.78		8.77	10.40
67	26.12	33.19	19.80	25.49	15.70	20.80	8.57	11.42
69	24.90	31.08	19.76		15.25		8.64	10.00
72	27.89	35.35	19.76		15.25		8.64	10.00
74	24.35	30.68	19.71	25.64	14.80	19.50	8.71	11.79
76	23.40	29.74	19.68		14.61		8.37	11.00
78	21.40	27.25	19.68		14.61		8.02	10.41
81	24.76	31.12	19.66	25.18	14.43	18.57	7.29	9.71
83	20.31	25.88	18.55		13.39		6.53	8.87
85	21.66	27.23	18.55		13.39		5.83	7.86
88	21.41	27.02	17.45	22.45	12.34	16.24	6.42	8.88
90	21.79	27.51	17.38		12.56		6.15	8.42
92	21.38	26.87	17.38		12.56		6.11	8.26
95	21.40	26.73	17.32	22.11	12.78	17.06	6.91	9.54
97	22.95	28.89	17.28		13.11		6.07	8.26
99	22.06	28.02	17.28		13.11		6.63	9.06

Table C-22. Continued.

	Train 2							
	F1		**F2**		**F3**		**F4**	
Day	**Total**	**Aceq**	**Total**	**Aceq**	**Total**	**Aceq**	**Total**	**Aceq**
102	22.53	28.55	17.25	22.63	13.43	17.93	6.91	9.67
104	21.85	28.06	17.10		12.94		6.45	9.01
106	22.04	28.41	17.10		12.94		5.55	7.86
109	20.82	27.02	16.95	22.25	12.44	16.96	6.42	9.17
111	20.90	26.97	16.87		12.86		5.82	7.97
113	21.93	28.38	16.87		12.86		6.72	9.23
116	21.35	27.48	16.79	22.12	13.27	17.77	6.48	9.04
118	22.00	28.26	17.55		13.53		6.57	8.79
120	21.31	27.27	17.55		13.53		6.28	8.62
123	22.17	28.32	18.31	23.64	13.80	18.56	6.80	9.27
125	20.55	26.15	17.57		12.98		6.56	9.12
127	20.81	26.58	17.57		12.98		6.19	8.72
130	20.93	26.86	16.84	22.23	12.17	16.69	6.19	8.76
132	20.82	26.77	16.46		12.07		5.79	8.00
134	20.92	27.07	16.46		12.07		5.24	7.20
137	20.28	26.05	16.08	21.35	11.98	16.19	6.07	8.22
139	21.83	27.95	16.13		11.88		6.13	8.31
141	18.98	24.30	16.13		11.88		5.62	7.46
144	20.21	25.76	16.18	21.33	11.78	15.78	6.08	8.29
146	20.13	25.89	16.29		12.31		6.18	8.31
148	20.33	26.05	16.29		12.31		6.59	9.38
151	22.00	28.59	16.41	21.88	12.83	17.78	7.19	10.11
153	20.48	26.62	17.27		12.47		4.69	6.54
155	21.23	27.75	17.27		12.47		5.91	8.38
158	21.84	28.63	18.14	24.50	12.11	17.06	5.79	8.08
160	20.89	27.57	16.72		12.70		5.36	7.51
162	20.15	26.58	15.30	20.92	12.23	17.02	5.00	7.02

Table C-23. Acid and aceq concentrations (g/L) for Train 3. Red indicates missing data was interpolated or estimated.

	Train 3							
	F1		**F2**		**F3**		**F4**	
Day	**Total**	**Aceq**	**Total**	**Aceq**	**Total**	**Aceq**	**Total**	**Aceq**
0	26.71	39.58	17.88	27.42	10.34	15.93	10.02	15.44
2	20.86	31.13	15.53	23.32	12.98	19.27	5.04	7.79
4	21.18	30.62	16.49	24.07	10.91	15.84	5.81	8.24
6	22.38	31.23	16.28	23.26	13.40	18.80	6.26	8.71
8	21.67	29.58	18.35	25.71	12.32	17.12	7.86	10.67
11	22.97	31.17	18.16	25.11	16.85	22.69	12.28	15.97
13	25.45	34.09	21.19	28.43	17.52	23.31	12.39	16.55
15	26.56	35.15	24.64	32.64	17.87	23.79	11.64	15.30
18	27.16	35.89	22.72	29.96	20.23	26.49	12.01	15.55
20	24.63	31.78					8.00	10.56
22	29.08	36.93					8.00	10.56
25	29.81	37.66					8.00	10.56
41	28.87	35.87					8.50	11.22
46	25.58	31.76					9.00	11.88
48	26.31	32.62	24.29	30.31	17.59	22.39	9.22	11.97
50	28.67	35.40						13.00
53	26.84	33.43						13.00
57	24.07	30.19						13.00
60	23.03	29.08	21.37	27.89	13.35	17.72	10.36	14.77
62	25.17	32.48	21.03		15.00		9.63	10.40
65	25.24	32.30	21.03		15.00		9.63	10.40
67	22.59	29.24	20.70	26.59	16.65	21.81	8.91	11.53
69	23.71	30.24	19.83		15.78		8.56	10.00
72	24.22	30.82	19.83		15.78		8.56	10.00
74	21.14	26.78	18.97	24.27	14.92	19.21	8.21	11.43
76	19.60	24.80	18.55		14.69		7.90	11.00
78	19.05	24.17	18.55		14.69		7.59	10.39
81	18.94	24.22	18.13	23.37	14.46	19.42	8.12	11.39
83	17.82	23.14	16.96		13.78		7.07	10.30
85	18.02	23.55	16.96		13.78		7.16	10.43
88	17.41	22.95	15.80	21.28	13.11	18.60	7.97	11.49
90	17.34	22.94	15.52		12.74		7.19	11.05
92	17.39	23.25	15.52		12.74		6.99	10.64
95	16.65	22.46	15.25	21.05	12.36	17.99	8.19	12.27
97	17.08	23.04	15.73		12.49		8.20	12.13
99	17.41	23.89	15.73		12.49		7.33	11.08

Table C-23. Continued.

	Train 3							
	F1		**F2**		**F3**		**F4**	
Day	**Total**	**Aceq**	**Total**	**Aceq**	**Total**	**Aceq**	**Total**	**Aceq**
102	18.08	24.89	16.21	22.80	12.62	18.48	7.41	11.23
104	16.74	23.14	15.72		13.02		7.83	12.06
106	16.55	23.21	15.72		13.02		6.72	10.57
109	17.05	23.91	15.23	21.71	13.43	20.29	7.48	11.76
111	16.60	23.33	14.87		13.81		7.26	11.74
113	17.66	25.09	14.87		13.81		7.23	11.37
116	15.81	22.34	14.51	21.07	14.20	21.51	8.43	13.41
118	18.63	26.40	16.23		14.37		7.68	12.51
120	18.07	25.76	16.23		14.37		7.66	12.21
123	18.18	26.09	17.95	26.10	14.55	22.10	8.40	13.42
125	18.96	26.94	17.71		13.83		9.41	14.67
127	18.89	26.99	17.71		13.83		8.51	13.26
130	19.99	28.31	17.48	25.38	13.12	19.45	7.91	12.83
132	20.58	28.63	17.86		13.62		7.39	11.46
134	20.43	28.31	17.86		13.62		7.86	12.03
137	21.51	29.65	18.24	25.79	14.12	20.57	8.46	12.58
139	20.38	27.66	18.57		13.50		8.04	12.15
141	20.07	27.63	18.57		13.50		7.78	11.88
144	20.30	27.76	18.90	26.42	12.89	18.58	8.28	12.31
146	20.70	28.12	17.88		13.66		7.39	10.86
148	19.60	26.60	17.88		13.66		7.71	10.60
151	19.64	26.44	16.86	23.11	14.44	19.73	8.31	12.11
153	20.03	26.91	18.30		14.11		8.31	11.73
155	19.00	25.42	18.30		14.11		8.01	11.77
158	20.87	28.06	19.75	26.77	13.78	19.66	9.85	14.70
160	20.80	27.82	18.79		13.70		7.86	11.99
162	19.47	26.43	17.83	24.48	12.38	17.88	7.97	11.82

Table C-24. Acid and aceq concentrations (g/L) for Train 4. Red indicates missing data was interpolated or estimated.

Train 4								
	F1		F2		F3		F4	
Day	**Total**	**Aceq**	**Total**	**Aceq**	**Total**	**Aceq**	**Total**	**Aceq**
0	24.77	36.78	18.24	27.69	9.97	15.37	9.46	14.57
2	21.82	32.29	16.75	25.04	12.25	18.43	5.96	8.76
4	20.57	29.51	15.77	23.10	10.32	14.99	5.81	8.22
6	21.66	30.20	15.92	22.65	12.12	17.20	7.66	10.56
8	20.30	27.48	16.68	23.15	11.95	16.50	10.44	13.66
11	21.27	28.09	17.18	23.65	15.16	20.44	12.41	16.40
13	22.10	28.77	19.73	26.51	16.75	22.28	12.18	16.09
15			20.56	27.10	19.71	26.08	10.86	14.15
18	21.61	27.75	24.33	31.58	19.43	25.22	15.86	20.35
20							13.00	16.90
22							13.00	16.90
25	27.51	34.26					13.00	16.90
41							13.00	16.90
46							13.00	16.90
48			22.10	26.75	16.51	20.41	11.70	14.19
50	26.88	32.22						13.00
53	23.91	28.89						12.00
57	20.29	24.53						11.50
60	20.43	24.72	19.02	23.12	14.74	18.42	8.93	11.04
62	22.19	26.65	19.48		15.09		9.13	10.40
65	21.44	25.93	19.48		15.09		9.13	10.40
67	21.74	26.01	19.95	24.59	15.45	19.25	9.34	11.76
69	22.19	26.93	17.97		13.13		6.33	10.00
72	20.18	24.39	17.97		13.13		6.33	10.00
74	16.81	20.73	15.99	19.90	10.81	13.33	3.31	4.08
76	14.76	18.07	15.07		11.36		5.11	7.00
78	14.93	18.01	15.07		11.36		6.90	8.67
81	14.17	17.16	14.16	17.41	11.90	14.74	9.59	13.21
83	14.28	17.48	14.45		11.79		7.09	8.90
85	14.09	17.31	14.45		11.79		8.92	11.51
88	14.57	17.91	14.75	18.15	11.67	14.87	8.99	12.72
90	16.66	20.45	14.38		11.05		8.84	12.00
92	14.32	17.69	14.38		11.05		7.70	10.98
95	13.67	17.13	14.02	17.78	10.43	13.93	8.62	12.50
97	14.34	18.07	13.59		11.39		7.96	11.15
99	13.87	17.66	13.59		11.39		8.06	11.11

Table C-24. Continued.

Train 4								
	F1		F2		F3		F4	
Day	Total	Aceq	Total	Aceq	Total	Aceq	Total	Aceq
102	14.77	19.13	13.15	17.32	12.35	17.48	8.55	11.96
104	14.47	18.73	12.52		11.57		9.31	4.65
106	13.84	18.55	12.52		11.57		8.12	11.43
109	12.64	16.91	11.90	16.01	10.79	14.64	8.37	11.94
111	12.34	16.53	13.19		10.63		7.29	9.95
113	12.96	17.18	13.19		10.63		8.34	11.98
116	12.90	17.21	14.49	19.74	10.47	14.54	8.74	11.90
118	12.51	16.92	13.02		11.22		8.37	12.31
120	11.74	15.87	13.02		11.22		8.63	11.82
123	13.49	18.12	11.54	15.94	11.97	16.03	8.72	12.88
125	10.91	14.87	11.76		10.88		9.16	13.23
127	11.77	15.69	11.76		10.88		7.59	10.80
130	12.22	16.71	11.98	16.44	9.79	13.52	8.60	12.59
132	12.70	17.18	13.33		10.72		7.96	11.61
134	11.74	15.89	13.33		10.72		7.79	10.95
137	11.89	16.17	14.69	20.52	11.65	16.12	9.10	13.26
139	16.22	22.03	14.55		12.32		8.17	11.07
141	13.93	18.83	14.55		12.32		8.40	12.10
144	15.44	21.06	14.42	19.43	12.98	18.03	8.25	11.07
146	15.00	20.07	14.82		13.25		8.17	11.25
148	15.20	20.61	14.82		13.25		9.08	12.30
151	15.90	21.69	15.22	21.17	13.53	18.87	8.72	12.15
153	14.20	19.57	14.61		12.97		9.34	12.40
155	14.15	19.47	14.61		12.97		9.32	12.94
158	15.00	20.50	14.00	19.01	12.41	16.99	9.17	13.08
160	14.23	19.20	13.88		11.66		8.99	12.61
162	14.02	19.05	13.76	18.81	12.09	16.50	6.25	8.46

Table C-25. Acid and aceq concentrations (g/L) for Train P. Red indicates missing data was interpolated or estimated.

	Train P							
	F1		F2		F3		F4	
Day	Total	Aceq	Total	Aceq	Total	Aceq	Total	Aceq
0	24.08	35.65	16.27	25.24	10.21	15.74	10.04	15.47
2	19.24	28.61	15.78	23.49	11.71	17.56	5.42	8.18
4	20.53	29.89	16.55	24.06	10.67	15.46	6.22	8.85
6	19.19	27.36	16.87	24.02	12.34	17.36	6.46	8.91
8	22.18	31.44	17.28	24.18	12.06	16.71	6.63	9.04
11	21.28	29.97	17.60	24.43	14.93	20.58	6.66	8.95
13	24.11	33.30	17.30	23.69	11.13	15.12	6.98	9.37
15	23.76	32.76	18.19	24.80	13.60	18.32	8.01	10.57
18	23.12	31.86	21.87	30.07			10.77	14.05
20	22.78	30.36					10.00	13.00
22	25.03	33.04					10.00	13.00
25	24.96	31.57					10.00	13.00
41	29.41	38.08					11.00	14.30
46	24.50	31.97					11.00	14.30
48	25.33	33.00	23.92	30.96	18.77	23.95	12.79	16.44
50	26.73	34.44						15.00
53	26.08	33.76						14.00
57	28.52	38.03						12.00
60	29.07	38.03	22.60	29.45	15.95	20.95	7.74	10.29
62	27.27	35.50	20.96		15.49		7.39	10.40
65	27.53	36.09	20.96		15.49		7.39	10.40
67	24.45	31.05	19.31	24.98	15.04	19.46	7.04	8.98
69	24.63	31.21	18.29		14.12		8.17	10.00
72	17.32	20.97	18.29		14.12		8.17	10.00
74	19.88	25.52	17.27	22.07	13.19	17.29	9.30	12.47
76	18.85	24.00	17.35		12.54		6.90	8.80
78	19.69	25.14	17.35		12.54		6.90	9.00
81	20.84	26.80	17.44	22.44	11.88	15.09	7.16	9.26
83	19.91	25.98	16.55		12.01		6.47	8.57
85	19.49	25.18	16.55		12.01		6.72	8.94
88	19.60	25.56	15.66	20.25	12.14	15.77	7.12	9.32
90	19.70	25.53	15.86		12.25		7.06	9.47
92	19.86	25.22	15.86		12.25		6.98	9.35
95	19.83	25.26	16.07	20.59	12.35	16.07	9.07	12.03
97	20.16	25.37	17.02		12.22		7.72	9.70
99	22.69	28.39	17.02		12.22		6.16	8.26

Table C-25. Continued.

Train P								
	F1		**F2**		**F3**		**F4**	
Day	**Total**	**Aceq**	**Total**	**Aceq**	**Total**	**Aceq**	**Total**	**Aceq**
102	21.03	26.94	17.96	22.72	12.10	15.91	8.41	11.38
104	22.56	28.54	16.43		10.85		7.07	9.49
106	19.11	24.75	16.43		10.85		7.24	9.64
109	19.48	25.24	14.89	19.38	9.60	12.79	6.91	9.46
111	18.89	24.55	15.63		11.24			9.30
113	19.74	25.77	15.63		11.24		6.84	9.20
116	19.66	25.62	16.37	21.36	12.88	17.05	7.71	10.42
118	19.63	25.50	16.37		13.05		7.37	9.88
120	19.43	25.27	16.37		13.05		7.58	10.09
123	21.05	27.57	16.36	21.38	13.23	17.39	7.83	10.70
125	20.62	26.81	16.17		12.38		7.77	10.41
127	21.48	27.90	16.17		12.38		7.11	9.53
130	21.05	27.39	15.99	21.00	11.53	15.24	6.94	9.29
132	20.63	26.73	16.22		11.57		10.39	13.57
134	21.07	27.32	16.22		11.57		6.74	8.66
137	21.09	27.36	16.46	21.39	11.62	14.98	7.58	9.96
139	21.07	27.36	16.60		11.99		7.46	9.76
141	19.83	25.69	16.60		11.99		7.43	9.84
144	21.62	28.07	16.75	21.77	12.36	16.16	7.05	9.56
146	19.47	25.24	16.49		11.66		6.84	9.11
148	21.44	27.58	16.49		11.66		5.96	7.66
151	20.05	25.71	16.23	21.03	10.97	14.15	7.73	10.25
153	19.82	25.53	15.99		11.83		6.70	8.65
155	19.27	24.71	15.99		11.83		7.75	10.24
158	20.71	26.66	15.75	20.24	12.68	16.47	7.70	10.32
160	20.34	26.02	17.13		12.22		8.23	10.85
162	18.20	23.59	18.52	24.08	11.77	15.54	7.79	10.27

Table C-26. Steady-state acid spectrum profile for Trains 1, 2, 3, 4, and P.

		C2	C3	IC4	C4	IC5	C5	C6	C7
Train 1	**avg.**	10.06	5.70	0.11	1.82	0.16	1.79	0.66	0.68
	stdev	0.88	0.97	0.02	0.28	0.02	0.19	0.23	0.24
	mass %	48%	27%	1%	9%	1%	9%	3%	3%
Train 2	**avg.**	9.87	8.31	0.13	1.24	0.18	1.35	0.11	0.06
	stdev	0.88	0.56	0.02	0.17	0.03	0.26	0.05	0.06
	mass %	47%	39%	1%	6%	1%	6%	1%	0%
Train 3	**avg.**	7.30	6.82	0.16	1.65	0.14	2.42	0.24	0.06
	stdev	1.14	0.97	0.02	0.35	0.02	0.79	0.10	0.02
	mass %	39%	36%	1%	9%	1%	13%	1%	0%
Train 4	**avg.**	6.24	4.77	0.08	1.21	0.08	1.31	0.08	0.07
	stdev	1.27	0.82	0.04	0.29	0.02	0.51	0.01	0.04
	mass %	45%	34%	1%	9%	1%	9%	1%	1%
Train P	**avg.**	9.70	6.86	0.12	1.67	0.16	1.58	0.10	0.02
	stdev	0.75	0.74	0.02	0.24	0.03	0.20	0.07	0.04
	mass %	48%	34%	1%	8%	1%	8%	1%	0%

Table C-27. Nitrogen and carbon content data for Trains 1, 2, 3, 4, and P.

		Day 138				Day 162			
		N	**C**	**C_{acid}**	**C_{NA}**	**N**	**C**	**C_{acid}**	**C_{NA}**
Train	**Ferm.**	g N/100 g total	g C/100 g total	g C_{acid} /100 g total	g C_{NA} /100 g total	g N/100 g total	g C/100 g total	g C_{acid} /100 g total	g C_{NA} /100 g total
Train 1	**F1**	0.17	5.76	0.77	4.98	0.13	5.89	0.86	5.03
	F2	0.11	4.50	0.66	3.84	0.08	3.59	0.71	2.88
	F3	0.09	3.74	0.48	3.26	0.06	3.26	0.36	2.90
	F4	0.07	3.57	0.24	3.34	0.07	2.53	0.22	2.31
Tain 2	**F1**	0.08	7.14	0.83	6.31	0.04	4.66	0.84	3.82
	F2	0.12	4.54	0.70	3.84	0.09	3.42	0.68	2.74
	F3	0.09	3.49	0.53	2.96	0.09	3.00	0.55	2.44
	F4	0.10	3.72	0.27	3.45	0.10	3.24	0.23	3.01
Train 3	**F1**	0.06	6.60	0.92	5.69	0.03	5.12	0.82	4.30
	F2	0.06	5.10	0.82	4.28	0.05	3.61	0.79	2.82
	F3	0.13	4.42	0.65	3.77	0.09	2.87	0.57	2.29
	F4	0.09	3.06	0.40	2.66	0.09	2.76	0.38	2.39
Train 4	**F1**	0.04	6.78	0.48	6.30	0.02	4.58	0.57	4.01
	F2	0.03	6.34	0.65	5.68	0.02	3.69	0.61	3.09
	F3	0.05	3.79	0.52	3.27	0.06	3.26	0.54	2.72
	F4	0.12	2.80	0.43	2.37	0.05	2.88	0.28	2.60
Train P	**F1**	0.09	5.93	0.85	5.07	0.06	5.04	0.74	4.30
	F2	0.11	4.20	0.70	3.49	0.06	3.6	0.79	2.81
	F3	0.10	4.47	0.49	3.98	0.07	2.57	0.51	2.06
	F4	0.10	4.34	0.33	4.00	0.07	2.96	0.34	2.62

APPENDIX D

ACID CONCENTRATION DATA FOR INVESTIGATION OF THE OPTIMAL CARBON-NITROGEN RATIO AND CARBOHYDRATE-NUTRIENT BLEND FOR MIXED-ACID BATCH FERMENTATIONS

Table D-1. Acid concentration (g/L) (i.e., average of three replicates) for each batch fermentation.

	Day						
Label	**0**	**7**	**14**	**22**	**28**	**30**	**32**
100-C	1.93	4.31	4.91	5.2	5.2	4.4	5.5
100-52	2.00	5.06	5.22	5.5	5.6	4.2	6.0
100-30	2.08	4.06	4.13	4.6	5.1	5.1	5.4
100-5	2.01	3.31	3.23	3.7	4.2	4.1	4.4
93-C	3.23	9.05	17.02	19.6	20.7	21.8	22.0
93-50	3.28	16.44	22.70	23.7	23.9	24.7	26.3
93-30	3.25	17.92	25.59	27.6	27.6	28.5	30.2
93-15	3.43	8.66	11.80	18.5	20.9	25.7	23.9
93-5	3.29	7.14	8.54	8.7	8.8	7.8	9.2
86-C	4.51	15.27	20.37	21.9	23.1	22.2	23.1
86-30	4.65	15.18	21.53	23.4	23.7	23.0	24.9
86-15	4.62	10.83	16.00	21.7	22.8	23.6	25.1
86-5	3.89	8.75	9.59	10.1	10.5	9.6	11.1
80-C	5.75	17.33	20.75	21.9	21.0	23.8	23.3
80-25	5.67	16.94	21.46	23.4	23.5	23.0	24.6
80-15	5.62	12.57	18.43	20.9	22.7	23.1	24.1
80-5	5.63	10.55	11.14	12.1	12.6	12.4	13.7
75-C	6.81	17.09	20.82	22.4	22.8	22.6	24.4
75-15	6.84	13.92	18.29	22.3	23.4	23.1	24.2
75-5	6.60	11.68	12.46	13.3	13.4	14.2	14.3
70-C	7.63	17.84	20.79	23.3	24.3	24.1	24.8
70-15	7.96	14.51	18.98	23.2	23.8	23.8	25.3
70-5	7.77	13.23	14.01	15.0	15.8	17.0	16.8
65-C	9.11	17.53	22.11	23.3	24.7	24.4	25.6
65-5	8.84	14.40	15.02	15.5	17.0	17.0	18.1
60-C	9.78	18.24	22.75	24.7	26.1	25.6	26.4
60-5	9.96	15.26	16.55	17.0	17.8	16.8	18.8
50-C	12.46	18.25	21.16	22.7	23.5	24.7	24.9
50-5	11.87	17.83	18.52	18.7	19.0	19.9	20.4
0-C	24.86	30.16	29.50	29.7	29.6	29.8	30.3

Table D-2. Aceq Concentration (g/L) (i.e., average of three replicates) for each batch fermentation.

	Day						
Label	**0**	**7**	**14**	**22**	**28**	**30**	**32**
100-C	2.57	5.44	5.97	6.1	6.1	5.3	6.4
100-52	2.69	6.27	6.29	6.6	6.6	5.1	7.0
100-30	2.76	4.99	4.95	5.4	6.0	6.2	6.4
100-5	2.68	4.22	4.08	4.4	4.9	4.6	5.0
93-C	4.10	11.03	21.02	24.0	25.3	26.6	26.8
93-50	4.18	21.10	28.52	29.6	29.7	30.6	32.6
93-30	4.12	23.72	33.15	35.5	36.0	36.6	38.5
93-15	4.34	10.17	14.39	26.2	29.7	36.1	33.9
93-5	4.18	8.09	9.55	9.7	9.8	8.9	10.5
86-C	5.66	19.53	25.72	27.5	29.0	28.0	28.9
86-30	5.85	19.29	26.81	29.0	29.2	28.6	30.8
86-15	5.76	12.44	18.74	28.0	29.2	30.4	32.0
86-5	5.06	9.93	10.78	11.3	11.8	10.7	12.5
80-C	7.13	22.60	26.78	28.2	29.7	30.5	29.7
80-25	7.03	21.82	27.08	29.4	29.5	28.8	30.8
80-15	6.98	14.50	22.73	27.2	29.3	29.0	30.9
80-5	6.99	12.02	12.54	13.7	14.2	13.7	15.3
75-C	8.40	21.90	26.30	28.2	28.6	28.3	30.6
75-15	8.42	15.98	22.19	28.3	29.4	29.1	30.9
75-5	8.14	13.25	13.98	15.0	15.2	15.7	16.2
70-C	9.37	22.14	25.78	28.8	30.0	30.0	30.6
70-15	9.80	16.65	22.60	29.3	29.9	30.1	31.7
70-5	9.54	15.12	15.89	17.0	17.9	19.2	19.0
65-C	11.15	21.48	27.50	28.9	30.5	30.2	31.5
65-5	10.82	16.45	16.99	18.0	19.4	19.5	20.9
60-C	11.96	21.45	27.26	29.9	31.5	30.4	31.9
60-5	12.17	17.35	18.70	19.2	20.2	19.0	21.3
50-C	15.16	21.17	24.77	26.8	27.8	29.3	29.6
50-5	14.44	20.45	21.17	21.5	22.0	23.4	23.7
0-C	29.96	34.63	33.49	33.8	33.5	33.8	34.2

Table D-3. Final (i.e., Day 32) carboxylic acid product profile and average molecular weight.

Label	C2	C3	IC4	C4	IC5	C5	C6	C7	avg MW
100-C	62.1%	35.2%	0.0%	0.8%	0.0%	0.0%	0.0%	1.9%	65.58
100-52	69.1%	22.9%	0.0%	3.3%	0.0%	3.4%	0.0%	1.3%	65.65
100-30	63.9%	28.2%	0.0%	4.0%	0.0%	3.3%	0.6%	0.0%	65.93
100-5	73.9%	19.2%	0.0%	3.4%	0.0%	3.5%	0.0%	0.0%	64.07
93-C	55.6%	36.3%	0.7%	3.2%	0.8%	3.2%	0.3%	0.0%	66.63
93-50	55.3%	31.6%	0.6%	5.1%	0.6%	6.0%	0.6%	0.2%	67.39
93-30	53.1%	28.6%	0.6%	6.4%	0.6%	8.7%	1.3%	0.7%	68.64
93-15	42.9%	18.4%	0.8%	15.4%	0.5%	10.4%	9.6%	1.9%	74.13
93-5	76.2%	12.2%	0.4%	7.5%	1.0%	2.1%	0.0%	0.7%	64.32
86-C	55.5%	29.6%	0.8%	5.6%	0.7%	6.0%	1.1%	0.7%	67.71
86-30	56.6%	30.4%	0.8%	5.2%	0.7%	5.2%	0.8%	0.3%	67.22
86-15	57.3%	21.5%	0.9%	8.2%	0.5%	7.1%	2.6%	2.0%	68.53
86-5	78.6%	11.8%	0.7%	5.5%	1.2%	1.6%	0.0%	0.6%	63.72
80-C	55.1%	25.3%	0.9%	7.1%	0.9%	7.3%	2.0%	1.5%	68.62
80-25	57.1%	26.6%	0.9%	6.2%	0.9%	6.3%	1.3%	0.8%	67.70
80-15	57.5%	19.1%	1.0%	8.6%	0.7%	7.2%	3.7%	2.2%	69.03
80-5	79.7%	9.4%	0.3%	8.1%	1.0%	0.9%	0.0%	0.6%	63.59
75-C	57.3%	25.1%	1.1%	6.6%	1.1%	6.4%	1.4%	1.0%	67.86
75-15	59.6%	20.7%	1.0%	6.6%	0.8%	7.2%	2.0%	2.1%	68.03
75-5	77.7%	11.1%	0.8%	7.1%	1.2%	1.4%	0.0%	0.6%	63.95
70-C	60.0%	24.3%	1.2%	5.4%	1.2%	5.6%	1.2%	1.1%	67.22
70-15	61.7%	19.1%	1.1%	5.5%	0.9%	6.7%	2.2%	2.8%	67.79
70-5	77.4%	12.3%	1.1%	5.8%	1.5%	1.3%	0.0%	0.5%	63.88
65-C	61.3%	23.4%	1.2%	4.5%	1.4%	5.7%	1.2%	1.3%	67.07
65-5	74.4%	13.4%	1.3%	7.5%	1.8%	1.2%	0.0%	0.4%	64.43
60-C	65.0%	21.0%	1.3%	4.5%	1.4%	4.7%	1.0%	1.1%	66.28
60-5	76.6%	13.8%	1.4%	4.9%	1.8%	1.2%	0.0%	0.4%	63.98
50-C	68.4%	18.2%	1.5%	5.0%	1.6%	3.6%	0.9%	0.7%	65.62
50-5	72.1%	15.2%	1.7%	7.3%	2.1%	1.1%	0.0%	0.4%	64.80
0-C	75.7%	17.3%	1.9%	1.3%	2.7%	0.9%	0.0%	0.2%	63.82

Table D-4. pH (i.e., average of three replicates) for each batch fermentation.

				Day			
Label	**0**	**7**	**14**	**22**	**28**	**30**	**32**
100-C	7.83	6.26	6.38	6.34	6.33	6.41	6.46
100-52	7.84	6.58	6.66	6.71	6.79	6.83	6.69
100-30	7.93	6.63	7.15	7.16	6.92	6.77	6.89
100-5	7.94	7.31	8.52	8.72	9.02	8.82	8.86
93-C	7.65	6.06	5.77	5.88	5.79	5.67	5.77
93-50	7.70	5.63	5.75	5.80	5.55	5.49	5.54
93-30	7.77	5.65	5.70	5.75	5.44	5.40	5.48
93-15	7.78	7.67	6.84	6.47	5.94	5.77	5.92
93-5	7.84	8.86	8.68	8.52	8.77	8.66	8.72
86-C	7.52	5.57	5.72	5.83	5.55	5.56	5.66
86-30	7.65	5.69	5.71	5.83	5.58	5.58	5.64
86-15	7.65	7.20	6.18	5.94	5.83	5.74	5.88
86-5	7.77	8.70	8.65	8.49	8.72	8.60	8.64
80-C	7.53	5.76	5.77	5.83	5.61	5.68	5.75
80-25	7.74	5.73	5.77	5.91	5.73	5.73	5.77
80-15	7.81	6.77	6.07	6.01	5.73	5.82	6.14
80-5	7.66	8.82	8.71	8.53	8.68	8.50	8.59
75-C	7.41	5.84	5.92	5.96	5.91	5.83	5.95
75-15	7.65	6.85	6.20	6.06	6.02	5.96	6.09
75-5	7.69	8.82	8.65	8.46	8.63	8.51	8.61
70-C	7.45	5.95	6.03	6.06	6.01	5.95	6.05
70-15	7.68	6.74	6.13	6.23	6.05	6.03	6.12
70-5	7.79	8.78	8.67	8.80	8.62	8.45	8.54
65-C	7.50	6.04	6.10	6.13	6.10	6.04	6.11
65-5	7.72	8.76	8.66	8.79	8.64	8.50	8.58
60-C	7.45	6.14	6.15	6.11	6.11	6.07	6.19
60-5	7.60	8.71	8.57	8.64	8.63	8.52	8.55
50-C	7.47	6.78	6.45	6.48	6.39	6.30	6.45
50-5	7.64	8.68	8.61	8.80	8.68	8.45	8.57
0-C	7.71	7.91	7.89	7.99	7.90	7.74	7.85

Table D-5. Target, Day-0, and Day-32 carbon and nitrogen data (i.e., average of three replicates) for each batch fermentation.

	Target			Day 0					Day 32				
	N	C_{NA}	C_{NA}/N	N	C	C_{acid}	C_{NA}	C_{NA}/N	N	C	C_{acid}	C_{NA}	C_{NA}/N
Label	g N/100 g total	g C_{NA} /100 g total	g C_{NA}/g N	g N/100 g total	g C/100 g total	g C_{acid} /100 g total	g C_{NA} /100 g total	g C_{NA}/g N	g N/100 g total	g C/100 g total	g C_{acid} /100 g total	g C_{NA} /100 g total	g C_{NA}/g N
100-C	0.10	17.4	166.	0.04	4.56	0.08	4.49	107.	0.05	1.35	0.20	1.15	23.1
100-52	0.35	17.3	50.0	0.09	4.86	0.08	4.78	53.0	0.08	2.71	0.21	2.50	30.2
100-30	0.58	17.2	30.0	0.13	4.91	0.08	4.83	36.3	0.11	1.51	0.21	1.30	11.8
100-5	3.25	16.2	5.0	0.76	4.94	0.08	4.86	6.4	0.38	1.97	0.17	1.81	4.7
93-C	0.24	15.8	65.4	0.09	4.48	0.13	4.36	51.0	0.11	3.93	0.87	3.06	27.8
93-50	0.32	15.8	50.0	0.09	4.45	0.13	4.32	48.7	0.20	3.53	1.05	2.48	12.1
93-30	0.53	15.7	30.0	0.12	4.74	0.13	4.61	36.9	0.13	3.96	1.21	2.75	21.0
93-15	1.04	15.5	15.0	0.21	4.68	0.13	4.55	21.7	0.22	4.20	1.03	3.17	14.2
93-5	2.99	14.9	5.0	0.70	5.14	0.13	5.01	7.2	0.20	2.91	0.34	2.56	13.1
86-C	0.36	14.5	40.5	0.13	4.54	0.17	4.37	33.8	0.20	4.04	0.92	3.13	15.9
86-30	0.48	14.5	30.0	0.14	4.58	0.18	4.40	30.7	0.17	4.36	0.98	3.37	19.7
86-15	0.96	14.3	15.0	0.22	4.62	0.18	4.45	19.8	0.30	4.21	1.02	3.20	10.8
86-5	2.76	13.7	5.0	0.91	4.95	0.15	4.80	5.3	0.30	2.12	0.41	1.70	5.6
80-C	0.45	13.5	30.5	0.17	4.64	0.22	4.42	26.7	0.20	4.26	0.92	3.34	16.5
80-25	0.54	13.5	25.0	0.18	4.52	0.22	4.30	23.9	0.23	4.31	0.98	3.33	14.5
80-15	0.90	13.4	15.0	0.24	4.71	0.21	4.49	18.6	0.32	4.23	0.98	3.25	10.2
80-5	2.59	12.9	5.0	0.64	5.15	0.21	4.94	7.7	0.32	2.68	0.51	2.17	6.8
75-C	0.51	12.8	25.2	0.21	4.61	0.26	4.35	20.7	0.34	4.35	0.98	3.38	9.9
75-15	0.85	12.7	15.0	0.27	4.61	0.26	4.35	15.9	0.43	4.23	0.97	3.26	7.7
75-5	2.46	12.3	5.0	0.67	5.02	0.25	4.77	7.1	0.41	3.24	0.53	2.71	6.6
70-C	0.57	12.1	21.5	0.24	4.91	0.29	4.62	19.0	0.26	4.49	0.97	3.53	13.5
70-15	0.81	12.1	15.0	0.29	4.83	0.30	4.53	15.8	0.33	4.36	1.00	3.36	10.2
70-5	2.35	11.7	5.0	0.57	4.83	0.29	4.54	8.0	0.49	3.75	0.62	3.12	6.4
65-C	0.62	11.5	18.7	0.29	4.78	0.34	4.44	15.3	0.29	4.42	1.00	3.42	11.6
65-5	2.24	11.1	5.0	0.63	4.92	0.33	4.59	7.3	0.47	2.78	0.68	2.09	4.4
60-C	0.67	11.0	16.5	0.34	4.94	0.37	4.57	13.6	0.35	4.89	1.02	3.87	11.0
60-5	2.14	10.6	5.0	0.64	5.00	0.37	4.63	7.2	0.49	3.37	0.70	2.67	5.5
50-C	0.76	10.0	13.3	0.44	5.11	0.47	4.64	10.5	0.42	4.78	0.96	3.82	9.1
50-5	1.97	9.82	5.0	0.65	4.76	0.44	4.32	6.7	0.54	3.78	0.76	3.02	5.6
0-C	1.04	6.89	5.0	0.91	5.49	0.91	4.58	5.0	0.89	5.08	1.11	3.97	4.5

APPENDIX E

This appendix contains the MatLab code used to predict product concentration and substrate conversion.

```
clear all
close all
global so taus a1 b1 e1 f1 g1 h1
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

%Start Simulation
disp(['Program starts at: ', datestr(now)]);
tic;

VSLR_data=[4,6,8,10,12]';
LRT_data=[10,15,20,25,30]';
ACID = [];
CONVERSION = [];
VSLR_loop=12; %loop is for varying VSLR.
%To make map, set to lowest VSLR, otherwise, set to specific VSLR
while VSLR_loop<12.1 % if want loop, set to highest VSLR
   LRT_loop=10;   %loop is for varying LRT.
   %To make map, set to lowest LRT, otherwise set to specific LRT
   while LRT_loop<30.01 %if want loop, set to highest VSLR

      %%Basic parameters for Fermentation
      stages=4; %Fermentor stages
      so=0.69; %Aeq selectivity (gAEQ/g VS digested)
      holdup =2.0; %ratio of liq to solid in wet cake (g liq/gVS cake)
      moist =.06; %ratio of liquid to solid in feed (g liq/gVS cake)
      SQ =1.0;
      ratio=0.77; %phi ratio of g total acid to g AEQ
      loading = VSLR_loop;
      tauloverall = LRT_loop;
      vol=[.48,.28,.28,.28]'; %Liquid volime in each fermentor
      totvol=sum(vol);
      liquidfeed = totvol/tauloverall;
      nnotreal = [169,214,214,214]'; %VS concentration gVS/L (?in each fermentor?)
      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);

e1=0.044; f1=1; g1=0.033; h1=2.5; %CPDM parameters
%acd=22.3; % acd need to transfer into the Function M file
rmodel = @(x1,acid) e1.*(1-x1).^f1./(1+g1.*(acid.*ratio).^h1);
syms x1 acid
drmodel_1 = diff(e1.*(1-x1).^f1./(1+g1.*(acid.*ratio).^h1),x1);
drmodel = @(x2,acd2) subs(drmodel_1,{x1,acd},{x2,acd2});

done = 0; %The index used to trace whether the condtion is satisfied
liqtoler = 0.05; %tolerance for Liquid flowrate 0.005
acidtoler = 0.1; %tolerance for acid concentration  0.02
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);  %CP 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*0.5);
    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')]);

%parameters for ODE45
options = odeset('RelTol',1e-3,'AbsTol', 1e-3);
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);  %calculate 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))*.4;  %why 0.4 here?
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_2)interp1(x,nhat,x_2);
```

```
          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_2)interp1(x,nhat,x_2).*x_2;
        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))/(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))*.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); %was chan3
          x_3=x;  nhat_3=nhat;
          F_3 = @(x_3)interp1(x,nhat,x_3);
          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_3)interp1(x,nhat,x_3).*x_3;
        Convrsn(i)= quad(F_32,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;
        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))*.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); %was chan4
            x_4=x;  nhat_4=nhat;
            F_4 = @(x_4)interp1(x,nhat,x_4);
            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; %25/nnot(i);
                else
                   nhatzero(i)= nhatzero(i) + (nnot(i) - nhattot)*0.7;
                end
            end

            F_42 = @(x_4)interp1(x,nhat,x_4).*x_4;
            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('Congratulations!  The simulation is successfully finished!')
      toc  %toc is used to check the whole time of the process

      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 + 5;
  end
  VSLR_loop = VSLR_loop + 2;
end

disp(['  acid levels = ',num2str(acid','%13.5f')]);
disp(['  convrsn levels = ',num2str(Convrsn','%13.5f')]);
disp(['  VSLR = ',num2str(VSLR_data','%13.5f')]);
disp(['  LRT = ',num2str(LRT_data','%13.5f')]);
disp(['  Acid levels = ',num2str(ACID','%13.5f')]);
disp(['           Conversions        =         ',num2str(CONVERSION','%13.5f')]);
```

VITA

Name: Aaron Douglas Smith

Address: Care of: Dr. Mark T. Holtzapple
Texas A&M University
Dept. of Chemical Engineering
232 Jack Brown Building (MS 3122)
College Station, TX 77843

Email Address: AaronSmith328@gmail.com

Education: B.S., Chemical Engineering, Mississippi State University, 2005
Ph.D, Chemical Engineering, Texas A&M University, 2011

Professional Interests: Bioprocessing, recycling, energy efficiency, leadership, leadership development

Personal Interest: whitewater kayaking, mountain biking, canoeing, camping, cooking, carpentry, volunteering

Publications:

Smith, A., Granda, C., Holtzapple, M., 2008. Biofuels for Transportation. in: M. Kutz (Ed.) Environmentally Conscious Transportation. Wiley, pp. 368.

Smith, A.D., Landoll, M., Falls, M., Holtzapple, M.T., 2010. Survey of Chemical Production from Lignocellulosic Biomass. in: K.W. Waldron (Ed.) Bioalcohol production: Biochemical conversion of lignocellulosic biomass. Woodhead Publishing Ltd., Cambridge, UK, pp. 560.

Smith, A.D., Holtzapple, M.T., 2010. The Slope Method: A Tool for Analyzing Semi-Continuous Data. Applied Biochemistry and Biotechnology, Epub. 22 Sept 2010.

Smith, A.D., Holtzapple, M.T., 2010. Investigation of Nutrient Feeding Strategies in a Countercurrent Mixed-acid Multi-Staged Fermentation: Development of Segregated-Nitrogen Model Bioresource Technology, 101, 9700–9709.