INVESTIGATIONS OF BIOMASS PRETREATMENT AND SUBMERGED FIXED-BED FERMENTATION

A Thesis

by

DANIEL MEYSING

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

MASTER OF SCIENCE

December 2011

Major Subject: Chemical Engineering

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Approved by:

Chair of Committee,	Mark T. Holtzapple
Committee Members,	Mahmoud M. El-Halwagi
	Cady R. Engler
Head of Department,	Michael V. Pishko

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ABSTRACT

Investigations of Biomass Pretreatment and Submerged

Fixed-bed Fermentation. (December 2011)

Daniel Meysing, B.S., Oklahoma State University

Chair of Advisory Committee: Dr. Mark T. Holtzapple

To improve the MixAlco process and biomass pretreatment, five studies were conducted. Three studies related to fermentation, whereas the other two investigated the effectiveness of shock tube pretreatment (STP) coupled with oxidative lime pretreatment (OLP).

In the first study, the constant-selectivity assumption used in the continuum particle distribution model (CPDM) was determined to be invalid. During a 32-day batch fermentation, selectivity increased from 0.10 to 0.40 g acid/g non-acid volatile solid (NAVS) digested. Future revisions to CPDM should incorporate a non-constant selectivity term.

In the second study, a revised procedure was developed to provide a more accurate determination of moisture content. Conventional drying at 105°C allowed product acids to vaporize with water, which introduced errors. Using the revised procedure, calcium hydroxide or sodium hydroxide was added to samples at a concentration of 0.01 g base/g sample, which retained acids in the sample. The mass of additional retained material closely matched that of the additional retained acid.

Three related studies involving biomass pretreatment were performed. In the first, recommended parameters for pretreating sugarcane bagasse with OLP and STP were determined. Recommended OLP parameters were 130°C, 6.9-bar O_2, and 2-h duration. The effects of solids concentration, liquid fill volume, particle size, type of shotgun shell, number of shocks, and pretreatment order were investigated. Liquid fill volume, particle size, type of shotgun shell, and pretreatment order were significant variables, whereas solids concentration and number of shocks were not.

Recommended OLP parameters were used as a basis for an additional experiment. To simulate industrial-scale pile fermentation, fixed-bed batch fermentation of OLP + STP sugarcane bagasse was performed in 1-L PVC fermentors. Rubber mulch was used as a structural support material to prevent filter plugging, which had been reported in previous work. After 42 d, acid concentration reached 8 g/L with yield approximately 0.1 g acid/g NAVS fed. Poor fermentation performance was caused by short solid-liquid contact time and poor pH control.

A third biomass pretreatment experiment investigated the potential of pretreated corn stover as a potential ruminant feed. Five samples (raw, OLP, STP, OLP + STP, and STP + OLP) were analyzed for composition and *in vitro* digestibility. STP followed by OLP increased neutral detergent fiber (NDF) digestibility from 49.3 to 79.0 g NDF digested/100 g NDF fed. On an organic matter basis, STP + OLP corn stover plus water-soluble extractives had a total digestible nutrients (TDN) of 74.9, nearly reaching corn grain at 88.1.

DEDICATION

I dedicate this thesis to my family. You have always given me the guidance and support I needed, while allowing me to make my own decisions.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Mark T. Holtzapple, for the guidance and support he has given me. He provided many suggestions and comments, which greatly improved my ability to complete this work.

I would also like to thank other members of Dr. Holtzapple's group: Dr. John Dunkleman, Dr. Melinda Wales, Dr. Andrea Forrest, Dr. Aaron Smith, Dr. Matt Falls, Kristina Golub, Michael Landoll, Sebastian Taco Vasquez, and Tyler Mann. Each of them have helped at crucial times, providing feedback and critical thinking. Several student workers, including Niroj Bhattarai, Pradnya Deshpande, Nishedh Khanal, Balaji Kothandaraman, Claudia Loboguerrero, Sirisha Medicherla, Kevin Mercy, and Lilibeth Orozco, have performed a large portion of the work. I would like to especially thank Pascale Chouinard-Dussault, who provided invaluable laboratory assistance and writing critiques.

Members of the department of chemical engineering staff have also helped a great deal. Jerry Bradshaw helped me think about safety aspects within the laboratory, especially relating to oxidative lime pretreatment. My appreciation is also extended to Randy Marek and several of his student workers who performed a great deal of work in the machine shop, fabricating, testing, and refurbishing equipment. I am greatly indebted for his assistance in all things mechanical and electrical.

NOMENCLATURE

AC	Ash content
Aceq	Acetic acid equivalent (g/L)
ADF	Acid detergent fiber
ADFIP	Average daily feed intake protein
ADIN	Acid detergent insoluble nitrogen
CP	Crude protein
CPDM	Continuum particle distribution model
dCP	Digestibility of crude protein
dEE	Digestibility of ether extract
DI H_2O	De-ionized water
dNDF	Ruminal and intestinal digestible neutral detergent fiber
DO H_2O	De-oxygenated water
DM	Dry matter
EE	Ether extract
ESTD	External standard (mixed carboxylic acid solution)
IADFIP	Indigestible average daily feed intake protein
ISTD	Internal standard (iso-valeric acid solution)
kf	Fractional rate of fermentation
LRT	Liquid retention time
m_A	Acid mass

MC	Moisture content
m_g	Gas mass
NAVS	Non-acid volatile solids
NDF	Neutral detergent fiber
NDFD	Neutral detergent fiber digestibility
NDFD48	48-h neutral detergent fiber digestibility
NDFIP	Neutral detergent fiber-insoluble protein
NDFn	Neutral detergent fiber adjusted for nitrogen
NDIN	Neutral detergent insoluble nitrogen
NFC	Non-fiber carbohydrate
n_i	Component *i* mole fraction
OLP	Oxidative lime pretreatment
OM	Organic matter
P	Pressure
r	Rate of fermentation
$\hat{r}$	Specific rate of fermentation
$\hat{r}_{pred}$	Predicted rate of fermentation
s	Batch selectivity
S_0	Initial substrate concentration
STP	Shock tube pretreatment
T	Temperature
t	Time

TDN	Total digestible nutrients
TDN_N	Total digestible nutrients, modified Weiss equation
TDN_W	Total digestible nutrients, Weiss equation
V	Gas volume
V_F	Gas volume corresponding to complete matter digestion
VS	Volatile solids
VSC	Volatile solids content
VSLR	Volatile solids loading rate
Y_C	Culture yield
Y_E	Exit yield
x	Conversion
α	Acetic acid equivalent (mol/L)
σ	Countercurrent selectivity
ϕ	Ratio of grams acid to grams Aceq
ρ_i	Component *i* density
λ	Lag time

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CHAPTER I

INTRODUCTION

As resources become more scarce in the 21st century, researchers are increasingly looking to produce fuels from biomass. Conventional non-renewable forms of energy do not replenish themselves sufficiently fast to meet consumption. Coal and oil required millions of years of natural processing to form, but they are being extracted from underground mines and wells within just a few centuries. In 2008, 6.6 billion kg of coal and 3.6 billion kg of crude oil were extracted, respectively (International Energy Agency, 2011a; International Energy Agency, 2011b). Each energy source has safety and environmental problems. In the United States alone, an average of 35 annual fatalities were caused by coal mining (2006–2010), with 48 occurring in 2010 (U.S. Mine Safety and Health Administration, 2011). Oil spills in Alaska (1991) and the Gulf of Mexico (2010) have demonstrated the environmental risks of oil.

The use of imported petroleum-derived transportation fuels is not sustainable for the United States. Approximately 41% of the petroleum imported into the United States comes from OPEC nations (U.S. Energy Information Administration, 2010). President George W. Bush stated that reliance on "unstable parts of the world" plays a key role in U.S. foreign policy and weakens American leverage (Bush, 2006). Without reducing the amount of imported petroleum products (transportation fuels, heating oil, and chemicals), the United States cannot significantly reduce its trade deficit. In 2009, U.S.

This thesis follows the style of Bioresource Technology.

net petroleum imports were 9.7 million barrels per day, amounting to 51% of total U.S. demand (U.S. Energy Information Administration, 2011). The North American Industry Classification System (NAICS) groups goods into distinct "codes." Using this system, import and export data can be easily grouped. Codes 2111, 2121, and 3241 collectively comprise crude oil and petroleum goods. In 2009, the amount of trade imports and trade deficits attributable to petroleum were 17.2% and 53.4%, respectively (U.S. International Trade Administration, 2011).

To create sustainable energy independent of conventional fuels, first-generation biofuels producers employed starch-based feedstocks. In the United States, this consisted of ethanol from corn grain. Commercialization of the corn-to-ethanol process has decreased corn grain available for food while increasing food commodity prices. The increased production of corn ethanol is one of several causes of increased corn prices (Rosegrant, 2008). From 2005 to 2010, the percentage of U.S. corn consumption devoted to biofuel production rose from 14% to 37% (U.S. Department of Agriculture, 2011a). From the early 1970s to the mid-2000s, the price of corn did not change dramatically from $2–$3/bushel. In the last 5 years, the price has increased dramatically to more than $7 per bushel (U.S. Department of Agriculture, 2011b). As a consequence, meat, dairy, and other agricultural commodity prices have increased as well. Increased food prices have caused food riots in some developing countries, including China, Egypt, Haiti, and Indonesia (Northoff, 2007). From 2000 to 2011, global population is expected grow by more than 800 million people (U.S. Census, 2011). To lessen malnutrition and starvation, crop yields must increase (Fedoroff & Cohen, 1999).

As transportation fuels, hydrocarbons have inherent advantages over ethanol. On a volumetric basis, gasoline contains about 50% more energy than ethanol. To be viable, costly ethanol infrastructure additions would be necessary. Ethanol is miscible with water and corrodes pipes.

In the United States, livestock consume 38% of the corn produced. Corn grain is used heavily as a livestock feed because it contains large amounts of highly digestible non-fiber carbohydrates (NFC): starch, sugar, and soluble fiber. The typical composition of corn grain is approximately 75% non-fiber carbohydrates (NFC), 10% crude protein (CP), 10% neutral detergent fiber (NDF), 3% fat, and 2% ash (Thornton et al., 1969). The calculated total digestible nutrients (TDN) value is generally over 85% (Chase & Hibberd, 1987).

The U.S. Department of Energy estimated the annual amount of waste lignocellulosic biomass from forest and agricultural resources to be 1.3 billion tons in the United States (Perlack et al., 2005). Because it contains lignin, which prevents access to cellulose fibers, mono-gastric animals, including humans, cannot digest significant amounts of lignocellulosic biomass. Cellulose fibers are substantially similar to starch (Figure 1-1); however, cellulose is undigestible in all vertebrates, except for ruminants which contain bacteria that cleave the β-1, 4 bond.

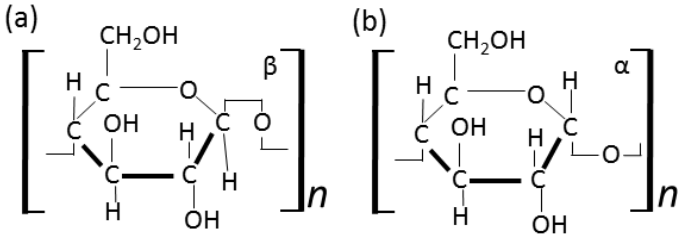

Figure 1-1. (a) Cellulose monomer and (b) starch monomer.

The MixAlco process produces hydrocarbon fuels from a variety of feedstocks, such as municipal solid waste, food scraps, sugarcane bagasse, sorghum, and waste office paper (Agbogbo, 2005; Aiello-Mazzari, 2002; Chan, 2002; Coleman, 2007; Domke, 1999; Fu, 2007; Ross, 1998; Thanakoses, 2002). In contrast to conventional petroleum, the MixAlco process emits less greenhouse gases. Further, it requires virtually no imported products, and does not use food crops as feedstock. Unlike ethanol, MixAlco hydrocarbons can be blended with conventional gasoline without a significant change in fuel energy value. The minimum gasoline selling price has been estimated at $2.56/gal (Pham, et al., 2010).

The first step in the MixAlco process is pretreatment, which allows agricultural residues, such as corn stover, to be subsequently fermented. Alternatively, pretreated biomass can be used as highly digestible constituents of ruminant animal feed. Previous research has demonstrated dramatic increases in enzymatic digestibility from oxidative lime pretreatment (OLP). OLP reduces biomass recalcitrance in two ways: (1) by cleaving acetyl groups on hemicellulose and (2) reacting with lignin to form soluble degradation compounds (Falls et al., 2011). Shock tube pretreatment (STP) increases

biomass digestibility, but the mechanism is unknown. Determination of optimal STP parameters helps refine equipment and improves basic knowledge.

The second step in the Mixalco process is mixed-culture fermentation. Naturally occurring organisms collected from marine environments convert lignocellulosic biomass to carboxylic acids. Using a methane analog, methane formation is inhibited. Fermentation yield is a key process metric.

Research is presented in the following chapters with these major objectives:

- determine suitability of the CPDM constant-selectivity assumption
- develop a revised moisture content analysis method
- determine OLP and STP parameters for highest sugarcane bagasse enzymatic digestibility and yield
- perform fixed-bed fermentation on OLP + STP sugarcane bagasse
- determine OLP and STP corn stover applicability as a ruminant animal feed

CHAPTER II

INVESTIGATION OF THE CPDM CONSTANT-SELECTIVITY ASSUMPTION

2.1 Introduction

Since 1996, continuous particle distribution modeling (CPDM) has been used to characterize Holtzapple-group fermentations (Agbogbo, 2005; Aiello-Mazzarri, 2002; Chan, 2002; Domke, 1999; Forrest, 2010; Fu, 2007; Loescher, 1996; Ross, 1998; Smith, 2011; Thanakoses, 2002). A continuum particle is defined as 1 g of volatile solid (VS) (Ross, 1998). CPDM predicts continuous countercurrent acid concentration and conversion for given volatile solids loading rate (VSLR) and liquid retention time (LRT) based on five 28-day batch fermentations. The five fermentations contain initial substrate concentrations of 20, 40, 70, 100, and 100 g/L, respectively. The fifth fermentation also contains an initial concentration of 20 g/L carboxylate salts (70% calcium acetate, 20% calcium propionate, and 10% calcium butyrate, by mass).

Previous research has demonstrated CPDM accuracy, which has been reviewed (Smith, 2011). Using a variety of substrates, CPDM predicts within 10% of the experimental acid concentration in 57% of studies and within 10% of the experimental conversion in 51% of studies.

CPDM assumes constant product selectivity (σ from Equation 2-20) throughout the 28-day batch fermentation. Further, it assumes the value is equal to the value determined in the countercurrent experiment. This simplifying assumption has no empirical data to support it. An improved CPDM model incorporating a non-constant

selectivity term would increase model accuracy. A previous attempt to improve CPDM prediction by correlating σ to VSLR reduced concentration and conversion error by about 50% (Aiello-Mazzarri, 2002).

Selectivity data are computed after completing batch fermentation, when final cake and product liquid are quantified. Therefore, intermediate selectivity data are not gathered during CPDM fermentations. In this chapter, a series of batch fermentations were completed to refine the CPDM model. Fermentations were run for varying lengths of time to gain insight into the suitability of the constant-selectivity assumption.

2.2 Materials and methods

2.2.1 Substrate

Office paper and chicken manure were used as substrates for this experiment. Office paper consisted of shredded recycled white office paper. Chicken manure was generously provided by Feathercrest Farms. To decrease variability, chicken manure was blended and dried at 105°C for 48 h. Following previous work, paper and chicken manure were used in an 80:20 mass ratio (Rapier, 1995).

2.2.2 Inoculum

Inoculum from Gulf Coast beaches in Galveston, Texas was used in all fermentations. This mixed culture of microbes is naturally occurring, and has been examined previously (Golub et al., 2011). Inoculum was collected by placing beach sediment from 0.5-m-deep holes in 1-L polypropylene bottles, and was mixed with de-oxygenated water (DO H_2O) for storage. See Appendix A for detailed collection procedure.

2.2.3 De-oxygenated water

To prevent oxygen exposure to anaerobic fermentation, DO H_2O was used. De-oxygenated water was prepared by boiling de-ionized water (DI H_2O), cooling to room temperature, and adding 0.275 g cysteine hydrochloride and 0.275 g sodium sulfide per liter of water. See Appendix B for detailed preparation procedure.

2.2.4 Methanogen inhibitor

To prevent methanogen growth, a 20 g/L iodoform/ethanol solution was added to fermentors. The solution was stored in a dark amber glass bottle in a refrigerator. Special care was taken to recap and refrigerate bottle immediately after use.

2.2.5 Fermentor design

Modified Beckman 1-L polypropylene bottles with two stir rods and a venting port were used as fermentors. Figure 2-1 shows fermentor components and an assembled fermentor. Stir rods were created by welding the ends of 1/4-in stainless steel tubes before bending them into the appropriate "S" shape. The rods were pushed through holes in a No. 11 rubber stopper. A crimp glass test tube was cut to produce a 3-in open tube. The non-crimp side was flared and the tube was pushed through a central hole drilled in the stopper. A rubber septum was forced into the glass tube and secured with an aluminum crimp seal. The bottle cap was modified by removing a portion of the inner radial area, so that the cap could hold the rubber stopper when screwed down. Fermentors were housed in a rolling incubator set to 40°C.

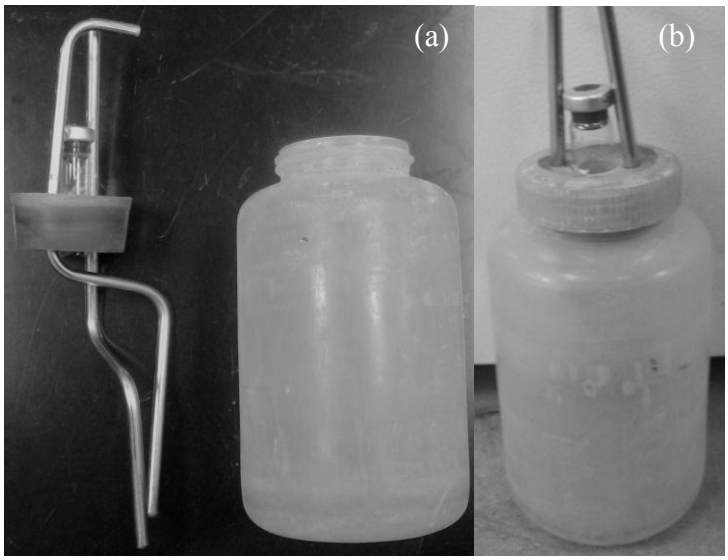

Figure 2-1. (a) Fermentor components; (b) assembled fermentor.

2.2.6 Calculations

2.2.6.1 Fermentation components

Fermentation material consisted of gas, volatile solids (VS), water, and ash. VS can be divided into product acids and non-acid volatile solids (NAVS). To calculate fermentation metrics, NAVS should be used in place of VS because product acids should not be considered as reactants (Smith, 2011). Along with N_2 gas added to purge fermentors, carbon dioxide and hydrogen were produced during fermentation. Product acids included acetic acid (C2), propionic acid (C3), *i*-propionic acid (IC4), *n*-butyric acid (C4), *i*-butyric acid (IC5), *n*-valeric acid (C5), caproic acid (C6), and enanthic acid (C7). Ash was expected to be conserved throughout fermentation. Figure 2-2 depicts the fermentation process. Notice that product acids were present at the beginning of fermentation (primarily from chicken manure).

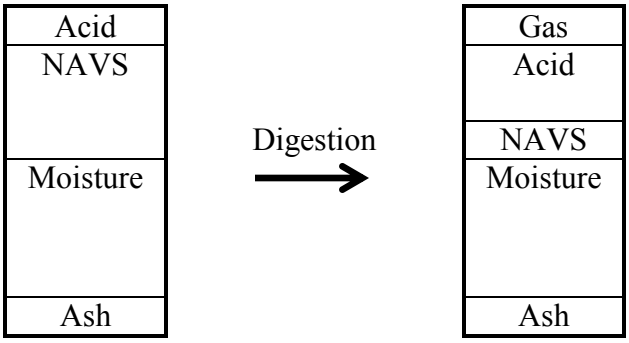

Figure 2-2. Schematic of fermentation digestion.

2.2.6.2 Acid and gas composition

Both acid and gas composition were measured using an Agilent 6890 Series gas chromatograph (GC) with helium as the carrier gas. Product acid composition was measured using a flame ionization detector and a 30-m fused-silica capillary column (J&W Scientific Model # 123-3232). From an initial temperature of 40°C, oven temperature was ramped up to 200°C at 20°C/min and held constant for 2 min. Samples were prepared by adding equal parts of sample, 3-M phosphoric acid, and iso-valeric acid standard (internal standard, or ISTD). Phosphoric acid was added to ensure protonation of carboxylic acids. The ISTD provided a calibration peak at a known concentration for *i*-valeric acid, which is not produced in mixed-culture fermentations. Intermittent external standard (ESTD) samples were also analyzed to allow calibration. The ESTD was purchased from Matreya, LLC (No. 1075). Gas composition was measured using a thermal conductivity detector and a 2.1-mm diameter, 4.6-m stainless steel-packed column. Inlet, detector, and oven temperatures were held constant for 10 min at 230°C, 200°C, and 200°C, respectively.

2.2.6.3 Gas volume

An inverted glass graduated cylinder connected to a plastic tube and vacuum pump was used to vent bottles and measure fermentor gas volume *V*. Figure 2-3 shows the gas venting apparatus. A syringe connected on the other end of the plastic tube was pushed through the fermentor septum, allowing gas to flow from the fermentor to the column, displacing the column of water. The vacuum pump was used to pull water up the graduated cylinder.

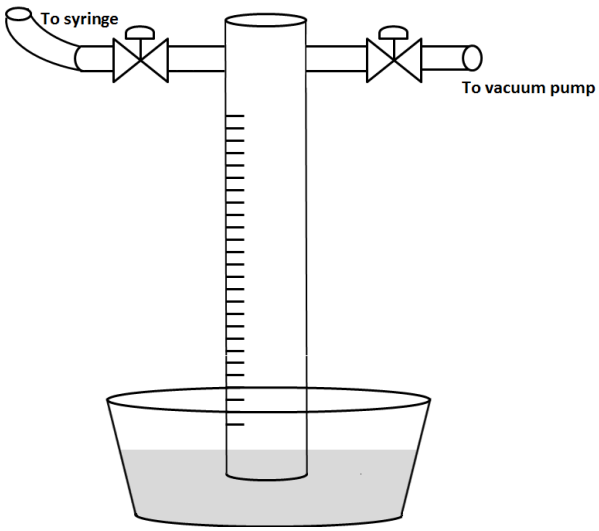

Figure 2-3. Gas venting apparatus.

Both the initial (H_1) and final (H_2) levels of water were recorded. Gas volume was calculated as

$$V = A(H_2 - H_1) \quad (2\text{-}1)$$

where:

V = gas volume (cm^3)

A = inner tube area (cm^2)

H_1 = initial water level (cm)

H_2 = final water level (cm)

The total gas mass m_g was calculated by

$$m_g = V_g \sum n_i \rho_i \quad (2\text{-}2)$$

where:

m_g = gas mass (g)

n_i = molar fraction of Component i in gas sample

ρ_i = density of Component i (g/cm^3)

2.2.6.4 Moisture, ash, and volatile solids content

Moisture content MC and ash content AC were measured using standard loss-on-drying methods (Sluiter et al., 2008a; Sluiter et al., 2005a). VS Content (VSC) was calculated by difference.

MC is given by

$$\text{MC} = \frac{w_2 - w_3}{w_2 - w_1} \tag{2-3}$$

where:

w_1 = weight of empty crucible (g)

w_2 = weight of crucible and wet sample (g)

w_3 = weight of crucible and dry sample (g)

In a similar manner, AC is given by

$$\text{AC} = \frac{w_4 - w_1}{w_2 - w_1} \tag{2-4}$$

where:

w_4 = weight of crucible and ashed sample (g)

Equation 2-4 gives AC on a wet basis. However, AC is generally reported on a dry basis from

$$\text{AC} = \frac{w_4 - w_1}{(w_2 - w_1)(1 - \text{MC})} = \frac{w_4 - w_1}{w_3 - w_1} \tag{2-5}$$

Volatile Solids Content (VSC) is calculated as the balance of material not attributable to MC or AC (wet basis) by

$$\text{VSC} = 1 - \text{MC} - \text{AC} = \frac{w_3 - w_4}{w_2 - w_1} \tag{2-6}$$

On a dry basis, VSC is the mass fraction of material not attributable to AC.

2.2.6.5 Mass balance

Mass exiting the system was calculated by summing mass sampled and mass remaining at fermentation end. Liquid samples were assumed to have a density of 1 g/mL. Mass entering the system was mass entering fermentation plus water of hydrolysis. Thus, mass closure was calculated by

$$\text{Mass closure} = \frac{m_g + \text{liquid sampled} + \text{final fermentor contents}}{\text{mass in} + \text{water of hydrolysis}} \tag{2-7}$$

Water of hydrolysis was calculated as the stoichiometric amount of water gained during digestion of NAVS to glucose. For the purposes of this calculation, NAVS was modeled as cellulose. Water of hydrolysis was calculated by

$$\text{Water of Hydrolysis} = \text{NAVS}_{\text{digested}} \left(\frac{18}{162}\right) \tag{2-8}$$

A mass balance was used to calculate $\text{NAVS}_{\text{digested}}$:

$$\text{NAVS}_{\text{digested}} = \text{NAVS}_{\text{in}} - \text{NAVS}_{\text{out}} \tag{2-9}$$

2.2.6.6 Fermentation performance

Fermentation metrics yield, conversion, and selectivity are regarded as a few of the most important measures of performance. Yield was described by two useful metrics: Culture yield Y_C and Exit yield Y_E.

Y_C refers to the yield obtained with respect to acid present in the substrate, and was given by

$$Y_C = \frac{m_A - m_{A_{t=0}}}{\text{NAVS}_{\text{in}}} \tag{2-10}$$

where:

$m_{A_{t=0}}$ = initial mass of acid (g)

m_A = final mass of acid (g)

Y_E is the amount of product in the system per unit mass of NAVS fed to the system. It was calculated by

$$Y_E = \frac{m_A}{\text{NAVS}_{\text{in}}} \tag{2-11}$$

Thus, Y_C measures the ability of the inoculum to produce product, and is discounted compared to Y_E.

Conversion x is the fraction of $\text{NAVS}_{\text{digested}}$ to NAVS_{in}.

$$x = \frac{\text{NAVS}_{\text{digested}}}{\text{NAVS}_{\text{in}}} \tag{2-12}$$

Selectivity s is the fraction of product acids per unit mass NAVS digested.

$$s = \frac{m_A}{\text{NAVS}_{\text{digested}}} \tag{2-13}$$

The product of x and s is Y_E. Following conventional chemical reaction engineering, s should be discounted by the initial acid concentration (Smith, 2011).

2.3 CPDM

Mathematica and Matlab programs have been used to predict acid concentration and conversion for continuous countercurrent fermentation at practical values of VSLR and LRT. Acid concentration is converted into a molar acetic acid concentration equivalent α by summing the weighting factor of each acid by its molar concentration:

α = acetic acid (mol/L) + 1.75·propionic acid (mol/L) + 2.5·butyric acid (mol/L) + 3.25·valeric acid (mol/L) + 4.0·caproic acid (mol/L) + 4.75·enanthic acid (mol/L) (2-14)

where:

α = acetic acid equivalent (mol/L)

Equation 2-15 was used to determine the acetic acid equivalent concentration Aceq for each batch fermentation on a g/L basis:

$$\text{Aceq} = 60.05 \times \alpha \tag{2-15}$$

Aceq is fit to an equation involving three constants (*a*, *b*, *c*), as shown in Equation 2-16.

$$\text{Aceq} = a + \frac{bt}{1 + ct} \tag{2-16}$$

where:

t = time (d)

The rate of fermentation *r* is found by differentiating Aceq with respect to time:

$$r = \frac{d(\text{Aceq})}{dt} = \frac{b}{(1 + ct)^2} \tag{2-17}$$

To determine the specific rate $\hat{r}$, the rate from the five batch fermentations is normalized by substrate concentration:

$$\hat{r} = \frac{r}{S_0} \tag{2-18}$$

where:

S_0 = initial substrate concentration (g NAVS/L)

$\hat{r}$ is used to determine constants (e, f, g, h) in Equation 2-19 for the predicted rate, $\hat{r}_{pred}$.

$$\hat{r}_{pred} = \frac{e(1-x)^f}{1+g[\phi\text{Aceq}]^h} \tag{2-19}$$

where:

x = conversion (g $NAVS_{digested}$/g $NAVS_{in}$)

ϕ = ratio of grams acid to grams Aceq

Conversion in Equation 2-19 is calculated by:

$$x(t) = \frac{\text{Aceq}(t) - \text{Aceq}(t=0)}{S_0\sigma} \tag{2-20}$$

where:

σ = selectivity (g Aceq/g $NAVS_{digested}$)

2.4 Experimental design

To examine product selectivity during batch fermentation, eight sets of three fermentations were terminated in constant 4-day intervals. Thus, the first set and eighth set were terminated after 4 and 32 days, respectively. Each fermentor was charged with 32 g shredded office paper, 8 g dry chicken manure, 6 g calcium carbonate, 0.5 g urea, 120 µL iodoform, 350 g DO H_2O, and 50 g Galveston inoculum. Fermentors were

placed in a rolling-bed incubator at 40°C. Gases were vented each day to prevent fermentor rupture. Every 2 days, 120 μL iodoform was added, pH was measured, and a 3-mL liquid sample was collected for acid analysis. To maintain an anaerobic environment, fermentors were purged with N_2 gas each time caps were removed. Every 4 days, the three terminated fermentors were centrifuged for 25 min using a Model J-6B Beckman centrifuge (3,297 × *g*, 4,000 rpm). Centrifuged liquids and solids were analyzed for mass, moisture, and ash content.

2.5 Results and discussion

Figure 2-4 shows average acid concentration for all fermentors. Error bars indicate standard deviation of all fermentors sampled that day. As expected, acid concentration remained low (below 5 g/L) for the first 8 d, increased dramatically during the next 8 d to about 12 g/L, and slowly increased thereafter. The final average acid concentration was 17.2 g/L, whereas the highest individual acid concentration was 20.4 g/L from Fermentor 8-3.

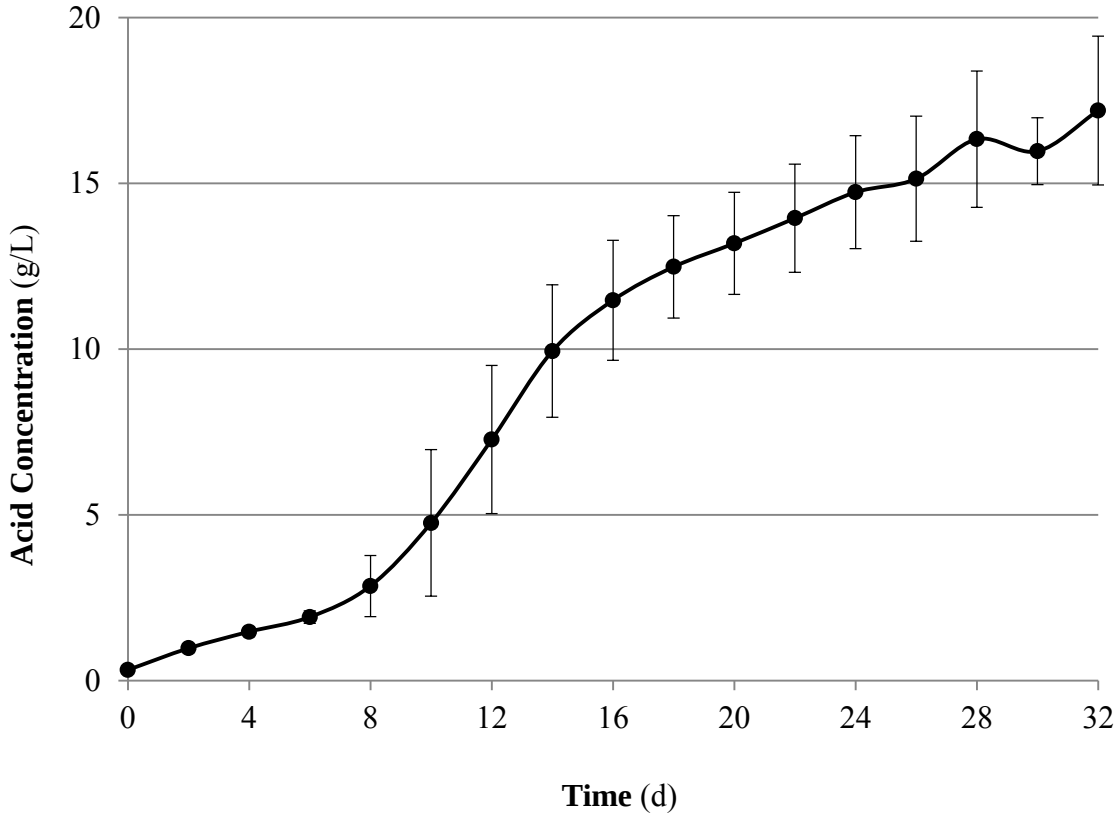

Figure 2-4. Average acid concentration profile. (Points are averages of all fermentations running, error bars represent ± 1 standard deviation.)

Table 2-1 shows set-averaged mass balance, conversion, exit yield, culture yield, and selectivity results. Mass balances were between 0.983 and 1.000. Mass balance decreased with time most likely because of mass loss during sampling. Conversion ranged from 0.169 g $NAVS_{digested}$/g $NAVS_{in}$ for Set 1 to 0.523 g $NAVS_{digested}$/g $NAVS_{in}$ for Set 7. In nearly all cases, conversion increased with time. Two exceptions did occur (Day 20 to Day 24, Day 28 to Day 32), but conversion never significantly decreased from one set to the next. The largest Y_E and Y_C, both from Day 28, were 0.192 g acid/g $NAVS_{in}$ and 0.187 g acid produced/g $NAVS_{in}$, respectively. Final yields were expected

to be the highest, but were insignificantly lower than those from Day 28. Selectivity increased steadily throughout time (Figure 2-5). Although selectivity was only 0.060 g acid/g $NAVS_{digested}$ after 4 days, it was 0.399 g acid/g $NAVS_{digested}$ after 32 days.

Table 2-1. Set-averaged fermentation metrics. (Averages ± 1 standard deviation.)

Time (d)	**Mass Balance**	**Conversion** ($NAVS_{digested}/NAVS_{in}$)	**Exit Yield** (g acid/$NAVS_{in}$)	**Culture Yield** (g acid produced/ $NAVS_{in}$)	**Selectivity** (g acid/ $NAVS_{digested}$)
4	0.998 ± 0.002	0.169 ± 0.059	0.009 ± 0.001	0.004 ± 0.001	0.060 ± 0.019
8	1.000 ± 0.002	0.204 ± 0.028	0.021 ± 0.009	0.016 ± 0.010	0.101 ± 0.031
12	0.995 ± 0.004	0.263 ± 0.072	0.068 ± 0.015	0.063 ± 0.013	0.259 ± 0.015
16	0.998 ± 0.003	0.409 ± 0.031	0.129 ± 0.012	0.125 ± 0.012	0.315 ± 0.009
20	0.995 ± 0.007	0.430 ± 0.017	0.148 ± 0.010	0.142 ± 0.011	0.344 ± 0.028
24	0.992 ± 0.006	0.415 ± 0.023	0.146 ± 0.012	0.143 ± 0.012	0.353 ± 0.011
28	0.988 ± 0.015	0.523 ± 0.019	0.192 ± 0.010	0.187 ± 0.010	0.367 ± 0.013
32	0.983 ± 0.004	0.466 ± 0.095	0.184 ± 0.030	0.181 ± 0.031	0.399 ± 0.029

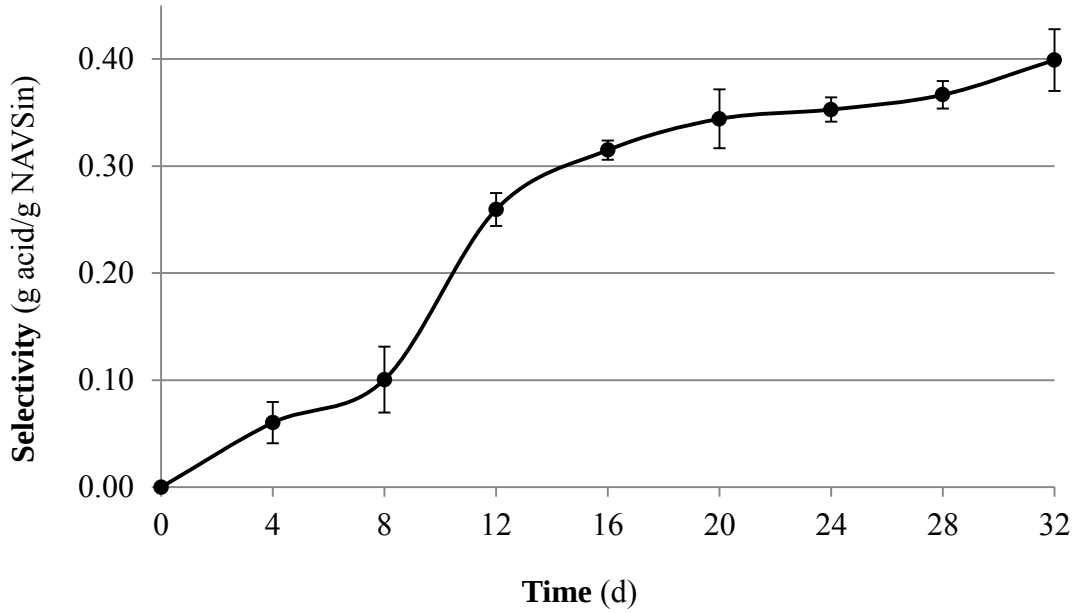

Figure 2-5. Set-averaged product selectivity. (Error bars indicate ± 1 standard deviation.)

2.6 Conclusion

To test the CPDM assumption that selectivity is constant over the duration of batch fermentation, eight sets of three fermentations were performed for 4 to 32 d. During the fermentation, selectivity increased from 0.1 g acid/g $NAVS_{digested}$ after 4 d to over 0.4 g acid/g $NAVS_{digested}$ after 32 d. The constant-selectivity assumption in CPDM should thus be revised to incorporate the time dependency of selectivity. Also, CPDM should be revised to include selectivity on an acid-produced basis rather than a total acid basis.

CHAPTER III

DEVELOPMENT OF AN ALTERNATIVE MOISTURE CONTENT ANALYSIS TECHNIQUE FOR MIXED-ACID FERMENTATION

3.1 Introduction

Accurate determination of fermentation moisture content (MC) is important for material accounting in the MixAlco process. MC directly affects conversion, presented in Equation 2-12. Conversion is a metric used in plant and fermentor design, and is equal to the fraction of feedstock digested during fermentation. Small errors in MC determination may lead to large errors in calculated conversion, because the non-moisture, non-ash fraction of samples is non-acid volatile solids.

Consider a hypothetical inaccurate measurement. Assume the actual MC of 1 g centrifuged fermentation liquids is 96%; however, because of inaccurate measurement, the calculated value is 98%. The percent error on the liquid fraction is only 2.08%, but error on the solid fraction is 50%.

$$\%\ \text{error (liquid)} = \frac{0.98\ \text{g} - 0.96\ \text{g}}{0.96\ \text{g}}(100\%) = 2.08\%$$

$$\%\ \text{error (solid)} = \frac{0.04\ \text{g} - 0.02\ \text{g}}{0.04\ \text{g}}(100\%) = 50\%$$

To analyze MC for samples containing acids, the NREL loss-on-drying procedure (Equation 2-3) should not be used because pH changes affect sample chemistry (Derikx et al., 1994; Sluiter et al., 2008a). Vaporized material is assumed to consist solely of water, whereas product acids are assumed to be retained. General acid-

base chemistry suggests that the NREL loss-on-drying procedure is unable to adequately retain acids during the drying process. Drying decreases pH and increases acid protonation because protonated acids freely vaporize simultaneously with water. The Henderson-Hasselbalch equation describes the relative relationship between pH and acid protonation/dissociation for a given substance.

$$pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right) \quad (3\text{-}1)$$

Thus, a change of 1 pH results in an order of magnitude change in the ratio of protonated acids to deprotonated acids. For common carboxylic acids (C2–C7), pKa values range from 4.74 to 4.89 (Merck Index, 2006; Solomons and Fryhle, 2004).

Since 1998, Holtzapple-group fermentations have been commonly buffered using calcium carbonate. Supersaturated calcium carbonate-buffered fermentations are mildly acidic. Table 3-1 displays reported low, average, and high fermentation pH values by Holtzapple group member (Agbogbo, 2005; Aiello-Mazzari, 2002; Chan, 2002; Coleman, 2007; Domke, 1999; Fu, 2007; Ross, 1998; Smith, 2011; Thanakoses, 2002).

Table 3-1. Historic Holtzapple-group calcium carbonate-buffered fermentation pH.

Group Member	High pH	Low pH	Average pH
Ross, Michael K.	6.20	5.85	6.03
Domke, Susan B.	6.80	5.60	6.16
Thanakoses, Piyarat	6.20	6.00	6.09
Aiello-Mazzarri, Cateryna	7.00*	5.50	5.91
Chan, Wen N.	7.00	5.50	6.08
Agbogbo, Frank	7.08	5.00	6.01
Fu, Zhihong	6.07	5.70*	5.84
Coleman, Stanley	6.50	5.20	5.68
Smith, Aaron	8.86	5.48	6.87

*Single outlier value was omitted and not factored into average.

Historically, Holtzapple-group fermentation pH has been low enough to allow a significant portion of product acids to remain in protonated form, thus readily vaporizing during MC analysis. Table 3-2 shows estimated acetic acid protonation/dissociation using the Henderson-Hasselbalch equation. The right-hand column provides an approximate protonated acid concentration for a hypothetical 20 g/L acetic acid solution.

Table 3-2. Predicted acetic acid state by pH using Henderson-Hasselbalch equation.

pH	[HA]/[A^-]	HA (g/L)
3.5	18.030	18.949
4.0	5.702	17.016
4.5	1.803	12.865
5.0	0.570	7.262
5.2	0.360	5.291
5.4	0.227	3.700
5.6	0.143	2.506
5.8	0.090	1.658
6.0	0.057	1.079
6.5	0.018	0.354
7.0	0.006	0.113
8.0	0.001	0.011
9.0	0.000	0.001

The objectives of this chapter were to:

- exhibit measurement error associated with the NREL loss-on-drying procedure on MixAlco fermentation samples
- develop a revised moisture content analysis method, which prevents acid vaporization yet resembles the NREL loss-on-drying procedure

3.2 Materials and methods

To accomplish the objectives, fermentation samples were analyzed for moisture content (Section 2.2.6.4). One of two basic compounds (calcium hydroxide or sodium hydroxide) was added to some samples. Addition of basic compounds was expected to increase pH, react with product acids, and thereby prohibit acid vaporization.

3.2.1 Fermentation material

Waste fermentation centrifuged liquids were collected from a 5-gal bucket used for discarding waste from various fermentation experiments. The broth composition was assumed to be constant throughout sampling for a single day.

3.2.2 Sample preparation

Figure 3-1 outlines the sampling regime. Waste centrifuged liquids were pipetted into pre-weighed 50-mL Erlenmeyer flasks. After recording the pH, the weight was recorded. Calcium hydroxide or sodium hydroxide was added to flasks at a pre-determined mass concentration of base to liquid. Bases were added at concentrations of 0.005, 0.010, 0.015, and 0.020 g base/g fermentation liquid. After recording the weight, the pH was recorded. Because of weight loss caused by pH measurement, the weight was recorded again. Flasks were then placed in an oven set to 105°C and dried for 24 h.

3.2.3 Reconstitution and storage

To prevent dried solids from absorbing moisture, samples were removed from the oven and immediately placed in a desiccator. They were weighed after cooling to room temperature. De-ionized water was added to each using a burette, and each sample was weighed. To prevent material loss, Parafilm was wrapped around flask rims, and

flasks were placed in a refrigerator for storage. To dislodge dried solids from the inner surface of the flasks, vigorous shaking was required. Once dried solids dissolved in water, solutions were decanted into 15-mL conical vials.

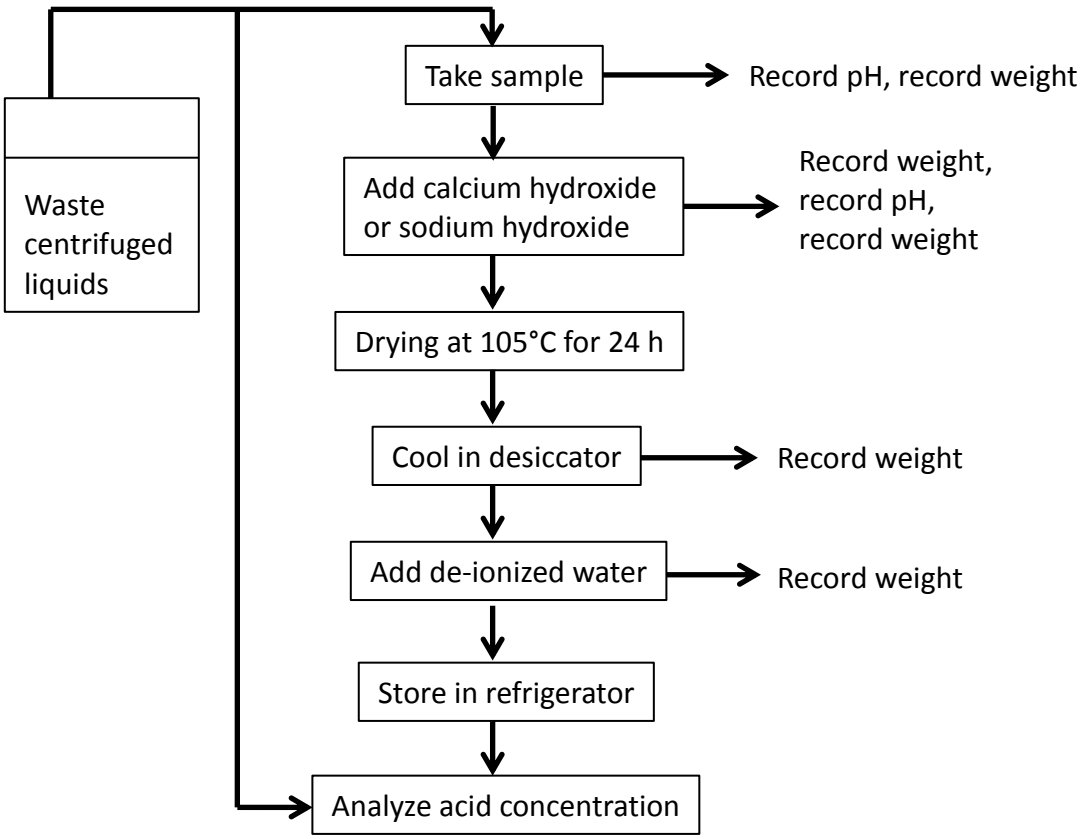

Figure 3-1. Moisture content analysis sampling regime.

3.2.4 Acid composition

Sample acid composition was determined by gas chromatography as described in Section 2.2.6.2.

3.2.5 Calculations

Sample weight was calculated by subtracting the flask weight from the flask and centrifuged liquids weight:

$$\text{Sample weight} = w_B - w_A \tag{3-2}$$

where:

w_A = flask weight (g)

w_B = flask and centrifuged liquids weight after pH measurement (g)

Base concentration was calculated as the mass concentration of base to mass of fermentation liquid:

$$\text{Base concentration} = \frac{w_C - w_B}{w_B - w_A} \tag{3-3}$$

where:

w_C = flask, centrifuged liquids, and base weight before pH measurement (g)

Calcium hydroxide and sodium hydroxide were assumed to not influence moisture content directly. Moisture content was calculated by

$$\text{MC} = \frac{w_D - w_E}{w_B - w_A} \tag{3-4}$$

where:

w_D = flask, centrifuged liquids, and base weight after pH measurement (g)

w_E = flask, dried solids, and base weight (g)

Acid retained is the ratio of acid in the reconstituted sample to acid in the stock solution.

$$\text{Acid retained} = \frac{(w_F - w_E)C_G}{(w_B - w_A)C_H} \quad (3\text{-}5)$$

where:

w_F = flask, dried solids, base, and water weight (g)

C_G = total acid concentration of sample (g/L)

C_H = total acid concentration of broth (g/L)

3.3 Results and discussion

3.3.1 pH

Figure 3-2 shows pH after base (calcium hydroxide or sodium hydroxide) addition. Base concentrations range from 0.005 to 0.020 g base/g fermentation liquid. Fermentation liquid pH was around 6.5. Increasing base concentration from 0 to 0.015 g/g fermentation liquid resulted in an increase in pH to 13. Further base addition did not increase pH.

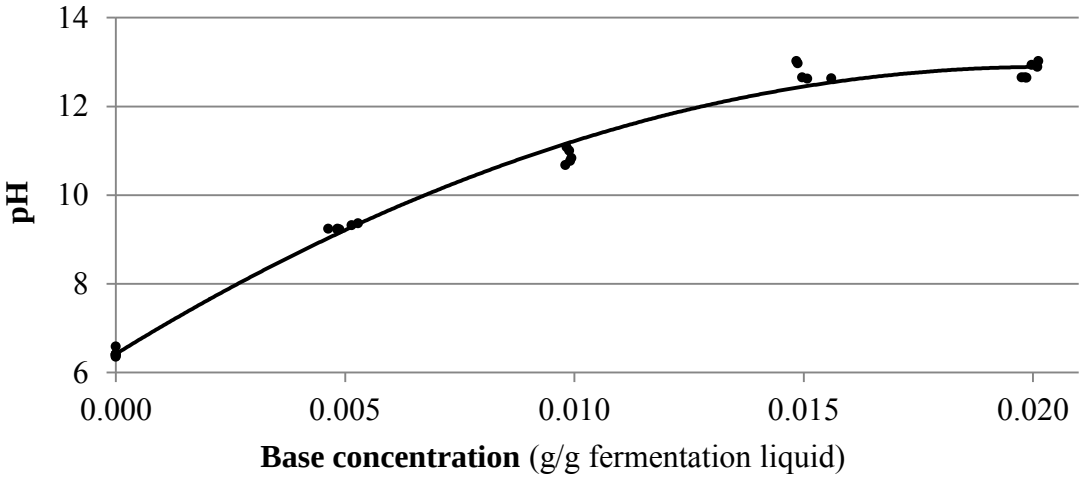

Figure 3-2. Liquid pH after base addition.

3.3.2 Moisture content

Figure 3-3 shows calculated moisture content by base concentration. Fermentation liquid MC was about 98%. Data points are averages of three measurements. Horizontal and vertical error bars represent 1 standard deviation for base concentration and moisture content, respectively. MC decreased to about 96.5% when 0.01–0.02 g base/g fermentation liquid was added. This trend was based on fewer product acids vaporizing with moisture during the drying process.

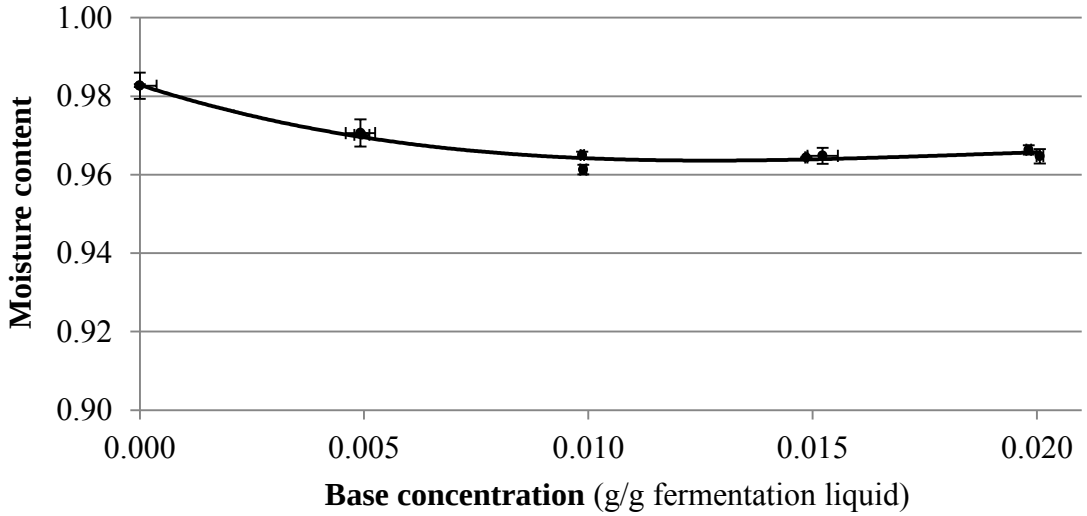

Figure 3-3. Effect of base addition on moisture content. (Error bars indicate ± 1 standard deviation.)

3.3.3 Acid retention

Figure 3-4 displays the retained acid profile for calcium hydroxide at concentrations between 0 and 0.02 g/g fermentation liquids. Data points are averages of three measurements. Horizontal and vertical error bars correspond to group concentration and acid retained standard deviations, respectively. Without addition of calcium hydroxide, less than 40% of the original acids were retained. Calcium hydroxide concentrations of 0.01 g/g fermentation liquids were adequate to retain nearly all acids.

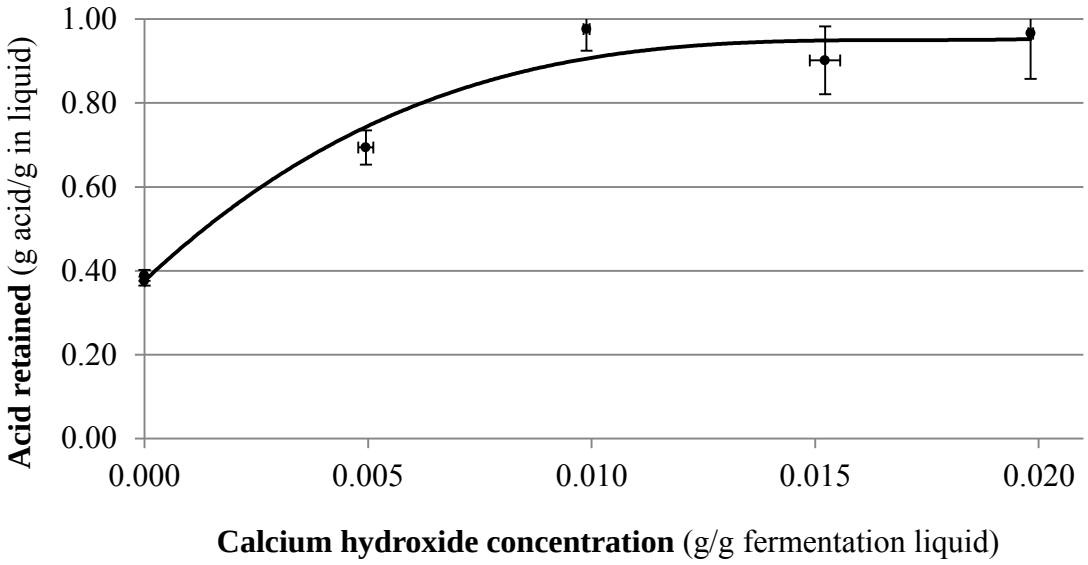

Figure 3-4. Acid retained profile for calcium hydroxide. (Error bars indicate ± 1 standard deviation.)

Figure 3-5 shows the acid retained profile for sodium hydroxide. Data points are averages of three measurements. Horizontal and vertical error bars correspond to the group concentration and acid retained standard deviations, respectively. Control points (0 g sodium hydroxide/g fermentation liquid) are identical to those in Figure 3-4. Sodium hydroxide concentrations above 0.01 g/g fermentation liquid were adequate to retain nearly all acids from the fermentation liquid.

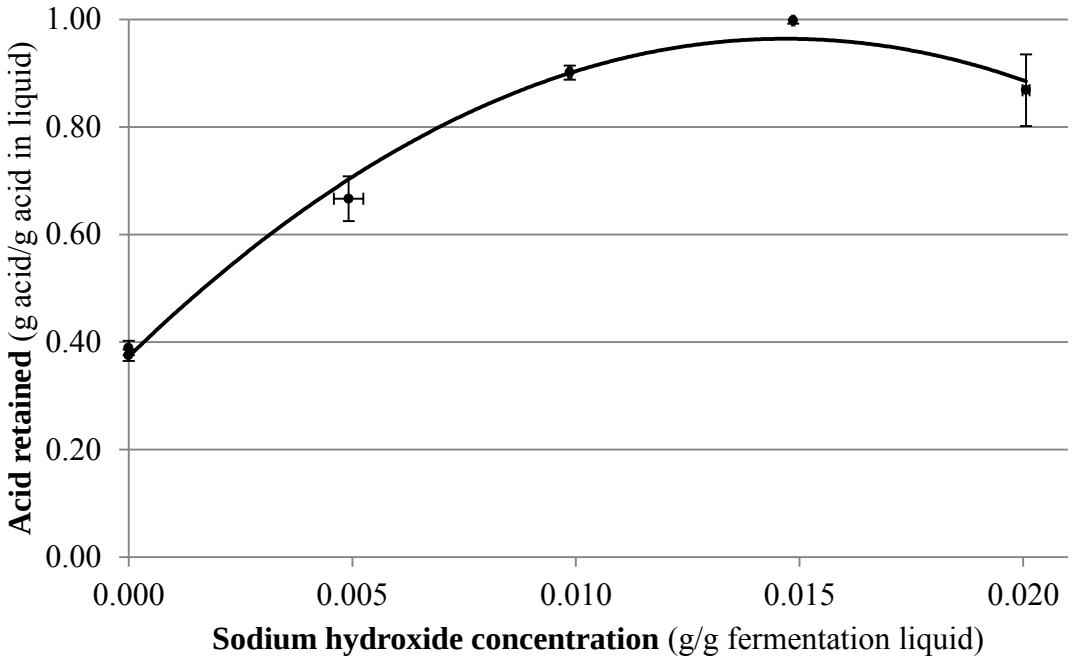

Figure 3-5. Acid retained profile for sodium hydroxide. (Error bars indicate ± 1 standard deviation.)

For the two sampling days, average acid concentration for sampled fermentation liquids were 19.5 ± 0.15 and 18.0 ± 0.11 g/L (averages of three measurements ± standard deviations). Interestingly, the increase in acid retained from 0.4 to 1.0 g acid/g acid in fermentation liquid is very close to the amount of decrease in moisture content from 98% to 96.5%:

Increase in acid retained = (1.0 – 0.4 g acid/g acid in liquid)(20 g acid/L)(0.02 L sample) = 0.24 g acid

Decrease in moisture = (0.98 – 0.965 g water/g sample)(1 g sample/mL sample)(20 mL sample) = 0.30 g

3.4 Conclusion

This study determined that fermentation samples can be accurately analyzed for moisture content using a modified loss-on-drying method by adding 1% calcium hydroxide or sodium hydroxide by weight. The addition of these two bases prevented acid vaporization, thereby promoting a quantitative split between moisture and the remainder of the sample: volatile solids, ash, and product acids. Moisture content decreased and acid retention increased by nearly the same mass when calcium hydroxide or sodium hydroxide was added. Using the NREL loss-on-drying method, about 60% of the sample product acids vaporized, which caused an erroneous increase of approximately 1.5% in moisture content.

CHAPTER IV

OXIDATIVE LIME AND SHOCK TUBE PRETREATMENT OF SUGARCANE BAGASSE

4.1 Introduction

Bagasse is a byproduct of sugar extraction from sugarcane. This fibrous material has been used to provide process heat for sugarcane factories. In 2008, 1.7 billion metric tons of sugarcane were produced globally (Food and Agriculture Organization, 2011). Such a large supply of low-value material could be pretreated to produce a higher-value product. Previous work has suggested that the recommended OLP duration and oxygen pressure parameters for maximizing sugarcane bagasse enzymatic digestibility and sugar yield are 2 h and 6.9 bar O_2, respectively. The recommended OLP temperature parameters for other types of biomass have been between 110 and 180°C (Ramirez, 2010). In addition, shock tube pretreatment (STP) has been demonstrated to further improve biomass digestibility when performed as a second pretreatment step after OLP (Falls and Holtzapple, 2011).

For this study, the goals were to:

- determine OLP temperature for highest enzymatic digestibility and sugar yield for sugarcane bagasse using a duration of 2 h and O_2 pressure of 6.9 bar
- determine STP parameters for highest enzymatic digestibility and sugar yield for sugarcane bagasse using best OLP parameters
- determine key STP variables

4.2 Materials and methods

4.2.1 OLP

OLP was performed on two different scales: (1) 145-mL reactors to determine optimal OLP reaction conditions, and (2) an 8-L reactor to produce large amounts of OLP bagasse using the determined optimal conditions for further STP investigations. Figure 4-1 shows the 145-mL reactors used for optimization, which consisted of 2-in-diameter stainless steel pipes with screwed caps. A port for oxygen was welded into the center of one cap, and connected to an O_2 supply line. Reactants were 8 g bagasse, 4 g calcium hydroxide, 120 mL DI H_2O, and 6.9 bar O_2. To provide heat and stirring, reactors were placed in a swinging arm assembly inside an oven.

Appendix D provides detailed operating procedures for 145-mL OLP. Loaded reactors were elevated to desired temperature and charged with O_2. After 2 h, reactors were removed and the oven was turned off. Subsequent neutralization and washing (Appendix G) removed residual calcium hydroxide and neutralized bagasse. Wet pretreated material was allowed to air dry in metal pans. Frequent stirring decreased drying time and prevented microbial growth.

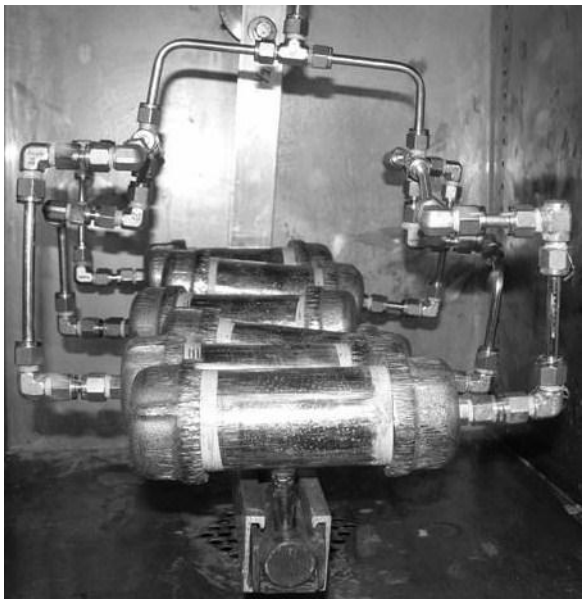

Figure 4-1. 145-mL OLP reactors.

In an 8-L reactor, larger-scale OLP for subsequent STP investigation was performed. Reactor contents were stirred with a magnetic drive fixed to an electric motor by a belt (see Figure 4-2). Based on a 2/3 working liquid volume, 310 g bagasse, 155 g calcium hydroxide, 4,650 g DI H_2O, and 6.9 bar O_2 were used. Appendix E provides the operating procedure. Reactants were loaded and manually stirred. Once heated to OLP temperature, the reactor was charged with O_2. Once pretreatment was complete, a chiller was used to shorten cool down time. After neutralizing excess calcium hydroxide and washing, the biomass was air dried.

Figure 4-2. 8-L OLP reactor.

4.2.2 STP

STP was performed in the shock tube apparatus (Figure 4-3). Appendix H provides detailed operating instructions. The shock tube is comprised of two carbon steel pipes connected by a 150-lb flange. The bottom pipe is a 20-in-long section of 4-in-diameter Sch. 80 pipe, and the top pipe is a 27.5-in-long section of 1-in-diameter Sch. 40 pipe. An 11-in-long conical section attached to the top pipe extends into the inner annular area of the bottom pipe. The conical section has an inner diameter of 0.88 in at the upper end, which increases to 3.56 in at the lower end. The shock tube was placed in a temperature-controlled water bath (25 ºC), and was loaded with bagasse and water. The top section was lowered onto the bottom pipe, and the shock tube was sealed. A 12-

gauge shotgun shell (Winchester Expert High Velocity 3 1/2-in, 1 3/8-oz steel BB shot) was placed inside the top opening of the top pipe and fired by releasing a steel plate firing pin onto the central metal surface of the shell. The flange was unbolted, and the top section of the shock tube was raised. The shock tube contents were placed in a product container and then filtered to remove lead shot and other shell remnants. Bagasse was then air dried in metal pans to uniform moisture content (<10%).

Figure 4-3. (a) Shock tube; (b) shock tube firing pin.

4.2.3 Calculations

All bagasse samples were analyzed for composition and enzymatic digestibility in triplicate. Measurements greater than 2 standard deviations from the mean were discarded.

4.2.3.1 Compositional analysis

Determination of moisture content, ash content, extractives, lignin content, glucan content, and xylan content was performed on untreated and treated bagasse. Moisture content and ash content determination are described in Section 2.2.6.4 (Appendix I and J, respectively). According to the NREL standard procedure, extractives content was determined (Appendix K; Sluiter, et al., 2005b). Both DI H_2O and ethanol were used as solvents. Exhaustive Soxhlet extraction solubilized bagasse extractives in solvent. Using a Rotavapor, the solvent was then boiled off under vacuum at 40°C. Extractives were measured by loss-on-drying:

$$\text{Extractives} = \frac{w_2 - w_1}{(w_3)(1 - \text{MC})} \tag{4-1}$$

where:

w_1 = flask weight (g)

w_2 = flask + extractives weight (g)

w_3 = biomass weight (g)

MC = moisture content

Extractives were assumed to be washed out during OLP and STP because of harsh temperatures (OLP) and washing (OLP and STP).

Using the NREL standard method, acid-insoluble lignin, glucan, and xylan contents were determined (Appendix L; Sluiter et al., 2008b). Bagasse samples underwent 1 h of concentrated acid hydrolysis (72% sulfuric acid) and 1 h of dilute acid hydrolysis (4% sulfuric acid). Using medium-porosity filtering crucibles, slurries were

then filtered. Filtering crucibles were dried at 105°C for 24 h and ashed at 550°C for 4 h.

Acid insoluble lignin (AIL) was calculated by

$$\text{AIL} = \frac{w_2 - w_3}{w_1(1 - \text{MC})} \tag{4-2}$$

where:

w_1 = sample weight (g)

w_2 = filtering crucible plus dry residue (g)

w_3 = filtering crucible plus ash (g)

Using calcium carbonate, the filtrates were neutralized. After centrifugation, a 1-mL syringe was used to draw and push a sample through a 0.2-µm syringe filter into an autosampler vial. Glucan and xylan content were analyzed by HPLC. An Aminex HPX-87P column, guard column, and refractive index detector were used. Conditions were 80°C with 0.6-mL/min flowrate, 20-µL injection volume, and 20-min run time. Sugar i content x_i was given by

$$x_i = \frac{\text{R}_{\text{SRS}_i} \times C_i \times \text{AHC}_i \times 87\ \text{mL}}{w_1 \times (1 - \text{MC}) \times 1000\ \text{mL/L}} \tag{4-3}$$

where:

C_i = HPLC-determined sugar concentration for Sugar i (g/L)

AHC_i = anhydro correction for component i (0.90 for glucan, 0.88 for xylan)

R_{SRSi} = recovery of sugar recovery standard for Sugar i

w_1 = sample weight (g)

4.2.3.2 Enzymatic digestibility and sugar yield

Using the NREL standard method, enzymatic hydrolysis was performed on raw and pretreated bagasse samples (Selig, et al., 2008). Cellulase and cellobiase enzymes with estimated activities of 59 FPU/mL and 288 CBU/mL, respectively, were used. Enzymatic digestibility was calculated as the mass of sugar digested during enzymatic digestibility per mass of sugar in the pretreated sample.

$$\text{Digestibility} = \frac{(V_{EH})(C_{glu,eh} \times \text{AHC}_{\text{glu}} + C_{xyl,eh} \times \text{AHC}_{\text{xyl}})}{\text{B}(1 - \text{MC})(x_{glu,p} + x_{xyl,p})(1000\ \text{mL/L})} \quad (4\text{-}4)$$

where:

V_{EH} = enzymatic hydrolysis total volume (10 mL)

$C_{glu,eh}$ = HPLC-determined glucose concentration after enzymatic hydrolysis (g/L)

$C_{xyl,eh}$ = HPLC-determined xylose concentration after enzymatic hydrolysis (g/L)

B = mass of bagasse loaded (g)

$x_{glu,p}$ = mass fraction glucan in the pretreated sample

$x_{xyl,p}$ = mass fraction xylan in the pretreated sample

Sugar yield was calculated as the mass of sugar digested during enzymatic hydrolysis per mass of sugar in raw bagasse.

$$\text{Yield} = \frac{(V_{EH})(C_{glu,eh} \times AHC_{glu} + C_{xyl,eh} \times AHC_{xyl})}{B(1 - MC)(x_{glu,p}/Y_G + x_{xyl,p}/Y_P)(1000\ \text{mL/L})} \quad (4\text{-}5)$$

where:

Y_G = pretreatment glucan yield (g glucan in pretreated/g glucan in raw)

Y_P = pretreatment xylan yield (g xylan in pretreated/g xylan in raw)

For OLP temperature variation, 72-h enzymatic hydrolysis was performed on wet bagasse at a cellulase loading of 5 FPU/g glucan in raw biomass. To measure maximum digestibility and yield, 72 hours was chosen. Because the mass of wet bagasse is unstable in open air, accurate loading was difficult. For STP studies, dry bagasse was used. To show relative rates of digestion, three time points (6, 24, 72 h) were selected. A higher cellulase loading of 15 FPU/g glucan in raw bagasse was used. In both studies, cellobiase loading was 60 CBU/g glucan in raw bagasse.

4.3 Experimental design

Eight independent OLP were performed at 10°C increments from 110°C to 180°C. Determination of the recommended temperature was based on combined glucose and xylose enzymatic digestibility and sugar yield. To provide protection from inaccuracy, each OLP was performed twice. Recommended OLP parameters were then used to produce a large quantity of OLP bagasse in an 8-L reactor.

Solids concentration, liquid fill volume, and particle size were assumed to be the most important variables affecting STP performance. Solids concentration is the amount

of bagasse added to the shock tube per amount of water. Previous research did not vary solids concentration from 0.05 g biomass/g H_2O (Falls, 2011). Practical lower and upper bounds were assumed to be 0.02 and 0.10 g bagasse/g H_2O. Liquid fill volume is the volume of water added. When closed, the shock tube lower pipe section volume is around 3 L. Previous work did not vary liquid fill volume from 2 L (Falls, 2011). Adding more than 2 L H_2O caused the slurry to overflow into and out of the upper pipe section. To test the effect of fill volume, 1 L was also used. Particle size is the size of individual bagasse fibers. Previous work has shown that particle size is an important variable in STP and OLP optimization. Reducing particle size increased pretreatment effectiveness (Falls, 2011). Particle sizes are given by mesh number. To further investigate the relationship between particle size and pretreatment effectiveness, two sizes were used (–4/+20 and –20/+40).

Table 4-1 shows the conditions for each STP. Additional nominal conditions were temperature (25°C), shotgun shell type (12-gauge, 3 1/2-in, BB shot), and number of shocks (1). A full-factorial experiment was performed involving solids concentration (0.02, 0.05, and 0.10 g bagasse/g H_2O), liquid fill volume (1 and 2 L), and particle size (–4/+20 and –20/+40). One STP with an alternative shotgun shell type (12-gauge, 3 1/2-in, 00 shot), one STP with three shocks, and one reverse-order pretreatment (STP on raw bagasse, then OLP) were also performed.

Table 4-1. STP testing matrix.

STP #	Solids Conc. (g bagasse/g H_2O)	Fill Volume	Particle Size
1	0.02	1 L	–4/+20
2	0.05	1 L	–4/+20
3	0.10	1 L	–4/+20
4	0.02	2 L	–4/+20
5	0.05	2 L	–4/+20
6	0.10	2 L	–4/+20
7	0.02	1 L	–20/+40
8	0.05	1 L	–20/+40
9	0.10	1 L	–20/+40
10	0.02	2 L	–20/+40
11	0.05	2 L	–20/+40
12	0.10	2 L	–20/+40
13[a]	0.10	2 L	–20/+40
14[b]	0.10	2 L	–20/+40
15[c]	0.10	2 L	–20/+40

[a]Used 00 shotgun shell
[b]Shocked three times
[c]STP first, then OLP

4.4 Results and discussion

4.4.1 OLP temperature variation

Figure 4-4 shows compositional analysis on raw and OLP bagasse on a dry matter (DM), raw basis. Thus, pretreatment mass fractions were multiplied by their respective pretreatment yields (g pretreated bagasse/g raw bagasse). Raw bagasse contained about 4% extractives, 2% ash, 24% acid-insoluble lignin, 41.5% glucan, and 22% xylan. Acid-soluble lignin and other structural sugars were measured, resulting in a mass balance of 0.94. Glucan and xylan masses remained relatively unchanged throughout the temperature range, totaling about 0.6 g sugar/g raw bagasse in most cases. As expected, acid-insoluble lignin content decreased after OLP. In some cases,

the mass of ash increased after OLP because of incomplete calcium removal during lime neutralization.

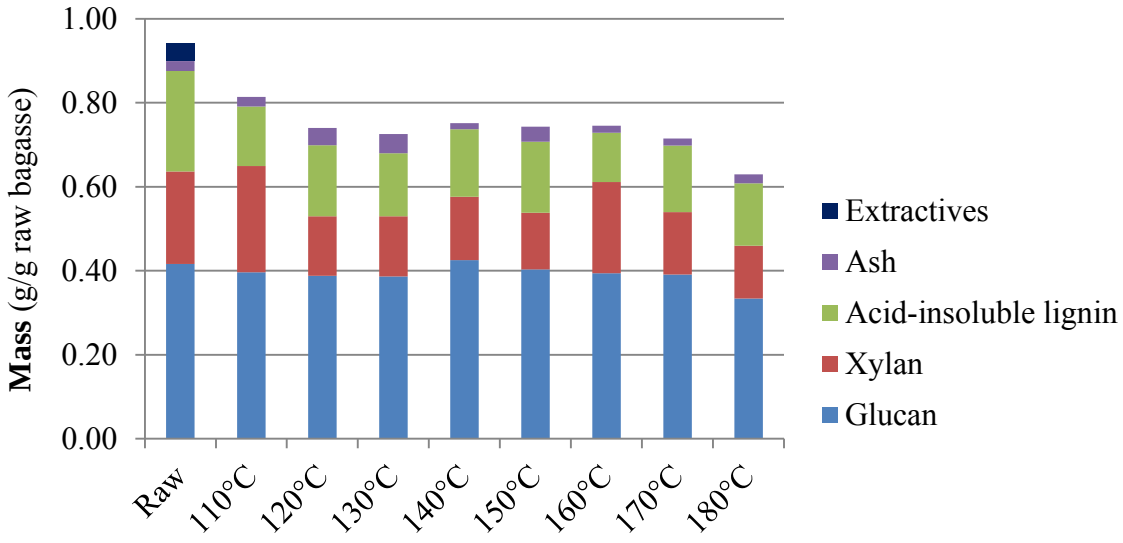

Figure 4-4. Raw and OLP bagasse compositional analysis (dry, raw basis).

Figures 4-5 and 4-6 show enzymatic digestibility and sugar yield for each OLP temperature, respectively. At 0.405 g sugar digested/g sugar in pretreated bagasse, OLP resulted in the most digestible bagasse at 130°C. The highest yield (0.337 g sugar digested/g sugar in raw bagasse) was also at 130°C. Thus, OLP was performed at 130°C for the remainder of this work.

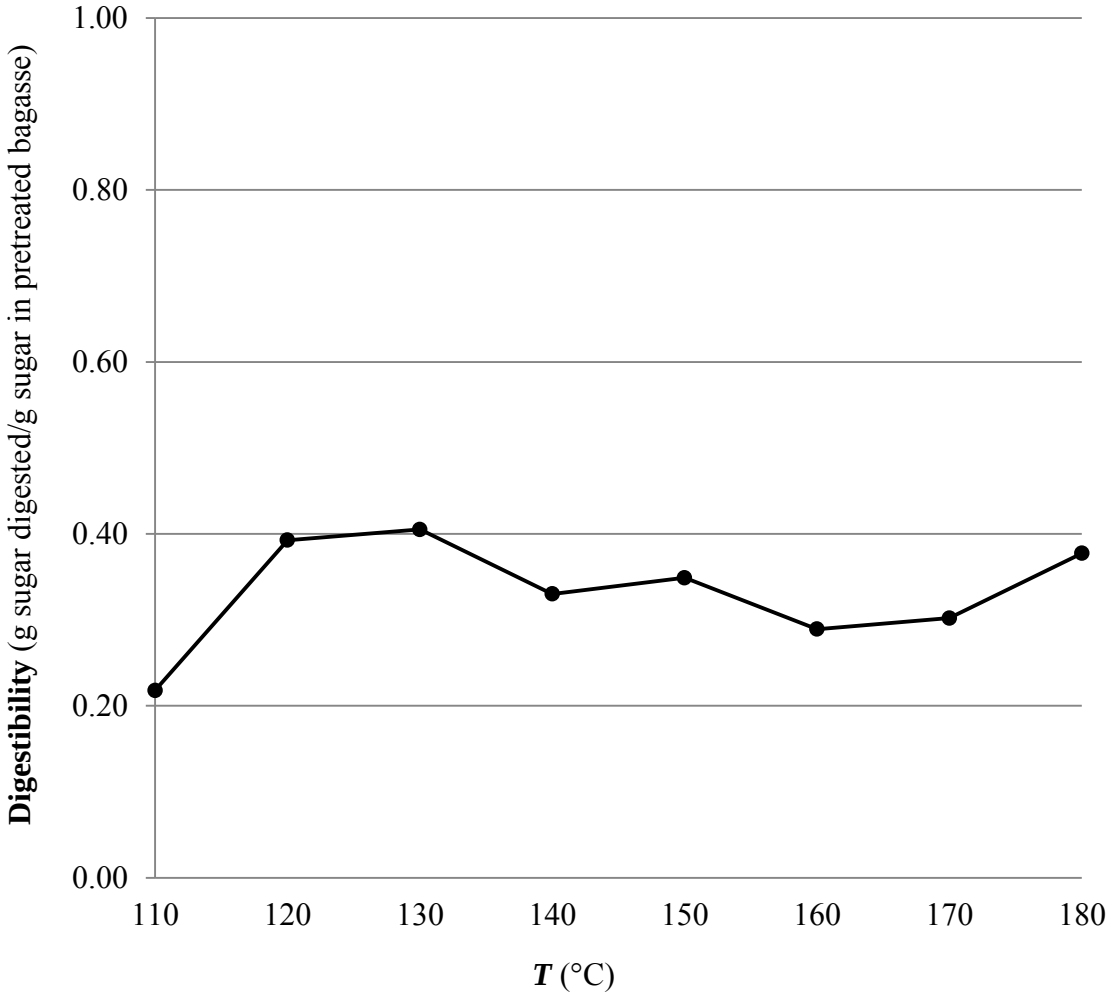

Figure 4-5. OLP sugarcane bagasse enzymatic digestibility by temperature. (Cellulase loading = 5 FPU/g glucan in raw bagasse.)

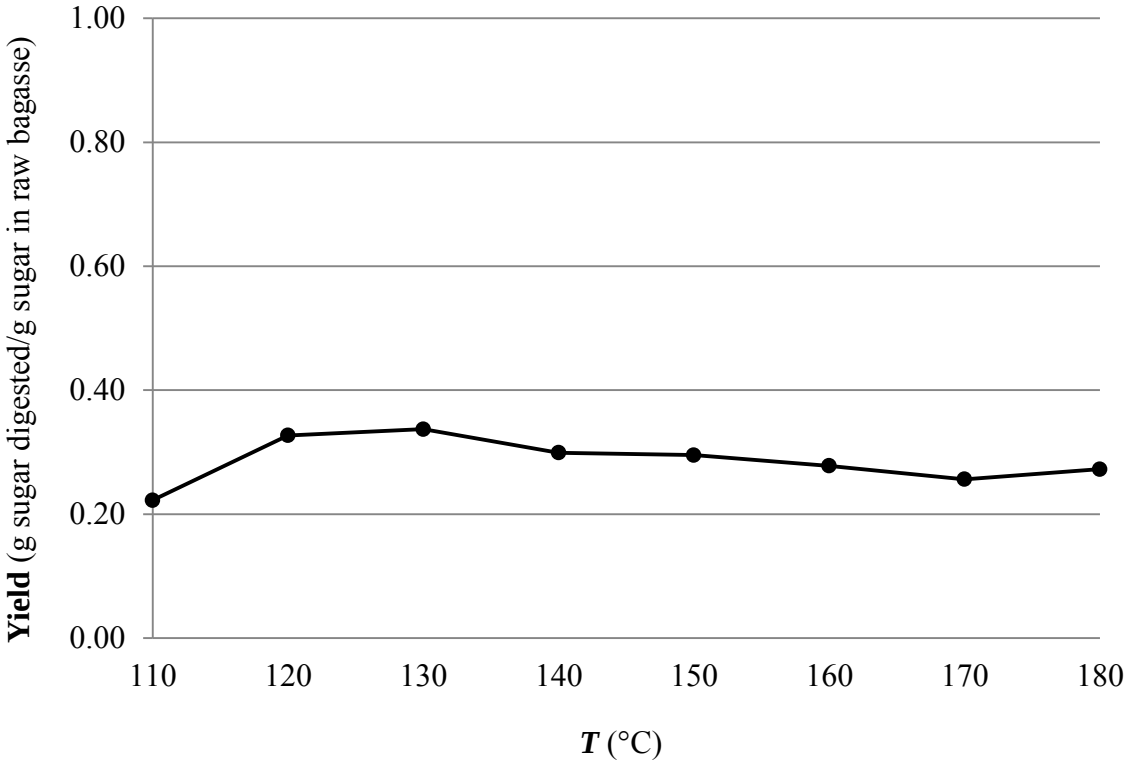

Figure 4-6. OLP sugarcane bagasse sugar yield by temperature. (Cellulase loading = 5 FPU/g glucan in raw bagasse.)

4.4.2 STP

4.4.2.1 Particle size

Figure 4-7 displays 6-, 24-, and 72-h enzymatic digestibility for –4/+20 and –20/+40 particle sizes. For comparison, raw and OLP bagasse are also shown. After 6 and 24 h, –20/+40 OLP + STP bagasse enzymatic digestibilities were significantly greater than for –4/+20 OLP + STP bagasse and OLP bagasse. At 24 h, enzymatic digestibility for –4/+20 OLP + STP bagasse was not significantly higher than for OLP bagasse. After 72 h, enzymatic digestibility for –20/+40 OLP + STP bagasse was

insignificantly higher than for –4/+20 OLP + STP bagasse and significantly higher than for OLP bagasse. Enzymatic digestibility for –4/+20 OLP + STP bagasse was significantly higher than for OLP bagasse. Digestibility for each of the three pretreated samples was at least 0.2 g sugar digested/g sugar in pretreated bagasse greater than for raw bagasse. STP significantly improved digestibility on OLP bagasse for the smaller particle size (–20/+40), and less significantly improved enzymatic digestibility for the larger particle size (–4/+20). During two of the three time periods, enzymatic digestibility was significantly greater for the –20/+40 sample than for the –4/+20 sample.

Figure 4-8 shows 6-, 24-, and 72-h sugar yield for –4/+20 and –20/+40 OLP + STP bagasse. Again, OLP + STP were more effective on smaller particle size bagasse; however, the two OLP + STP samples and the OLP sample were not as greatly separated. Yield was at least 0.15 g sugar digested/g sugar in raw bagasse higher for the three pretreated samples than for the raw sample.

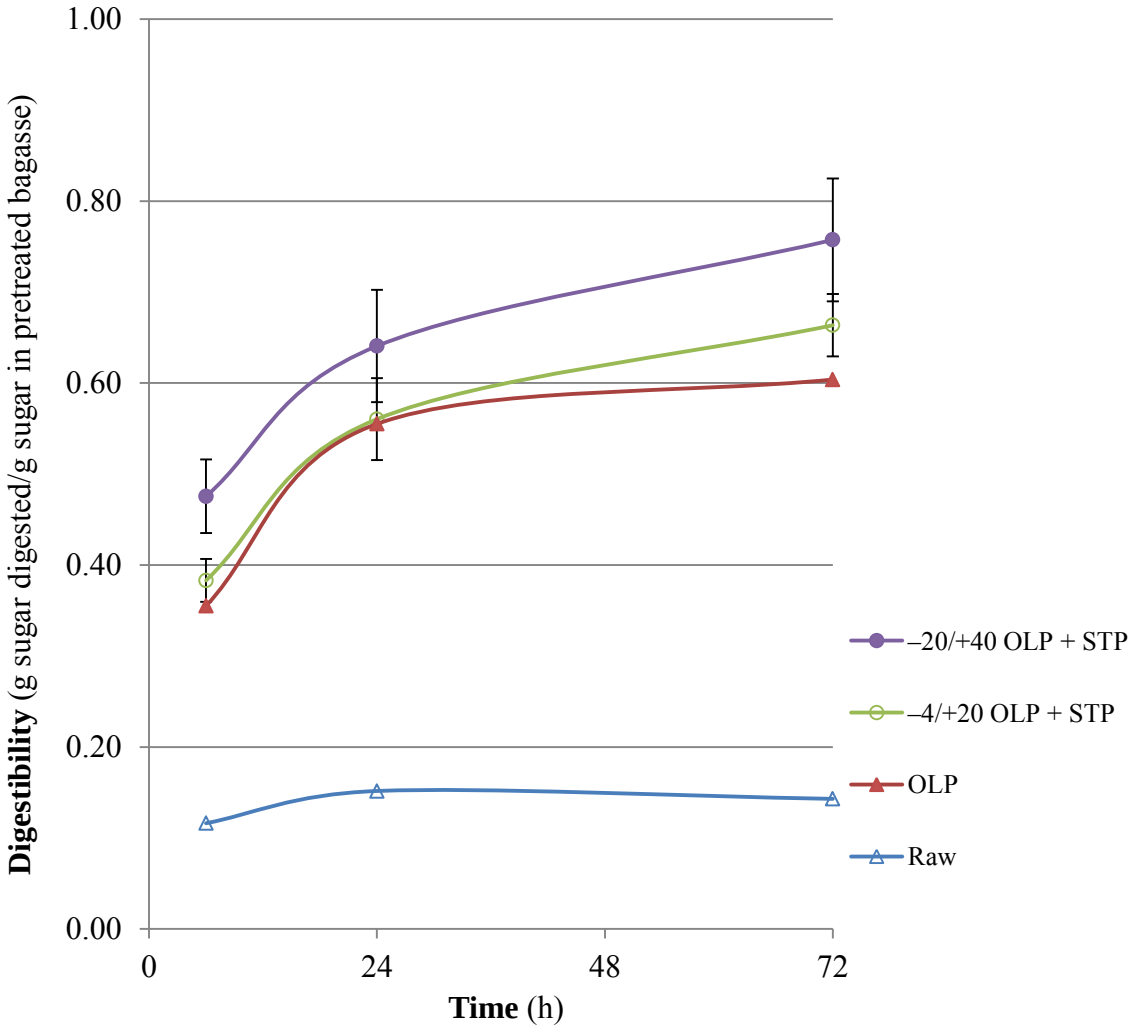

Figure 4-7. Enzymatic digestibility for raw, OLP, –4/+20 OLP + STP, and –20/+40 OLP + STP bagasse. (Cellulase loading = 15 FPU/g glucan in raw bagasse; error bars indicate ± 1 standard deviation.)

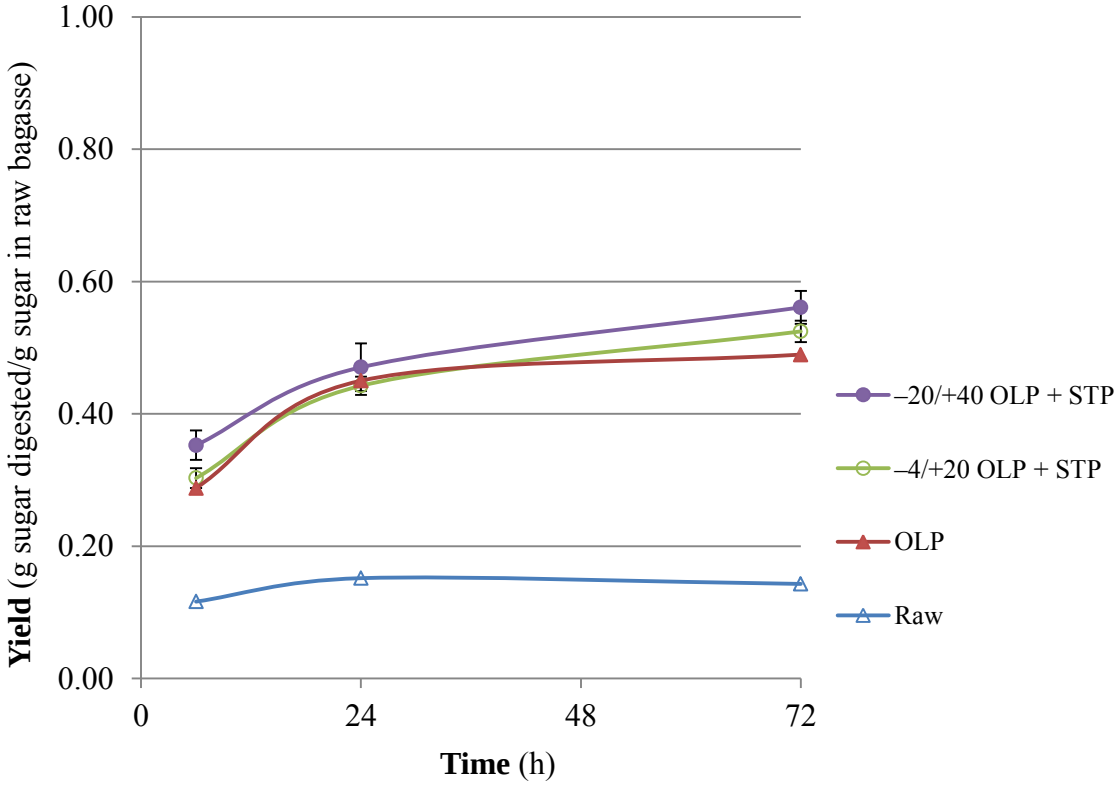

Figure 4-8. Sugar yield for raw, OLP, –4/+20 OLP + STP, and –20/+40 OLP + STP bagasse. (Cellulase loading = 15 FPU/g glucan in raw bagasse; error bars indicate ± 1 standard deviation.)

4.4.2.2 Solids concentration

Figure 4-9 shows 6-, 24-, and 72-h enzymatic digestibility for OLP + STP bagasse at 0.02-, 0.05-, and 0.10-g bagasse/g H_2O STP solids concentrations. Enzymatic digestibility for raw and OLP bagasse is also shown. For each time period, each solids concentration group had higher enzymatic digestibility than OLP bagasse; however, group-average 6-h (0.43, 0.43, and 0.43 g sugar digested/g sugar in pretreated bagasse), 24-h (0.58, 0.62, and 0.61 g sugar digested/g sugar in pretreated bagasse), and 72-h (0.69, 0.74, and 0.70 g sugar digested/g sugar in pretreated bagasse) were not

significantly different. Each time period, enzymatic digestibility for OLP bagasse was significantly lower than for one or two OLP + STP bagasse samples. Solids concentration did not substantially affect enzymatic digestibility.

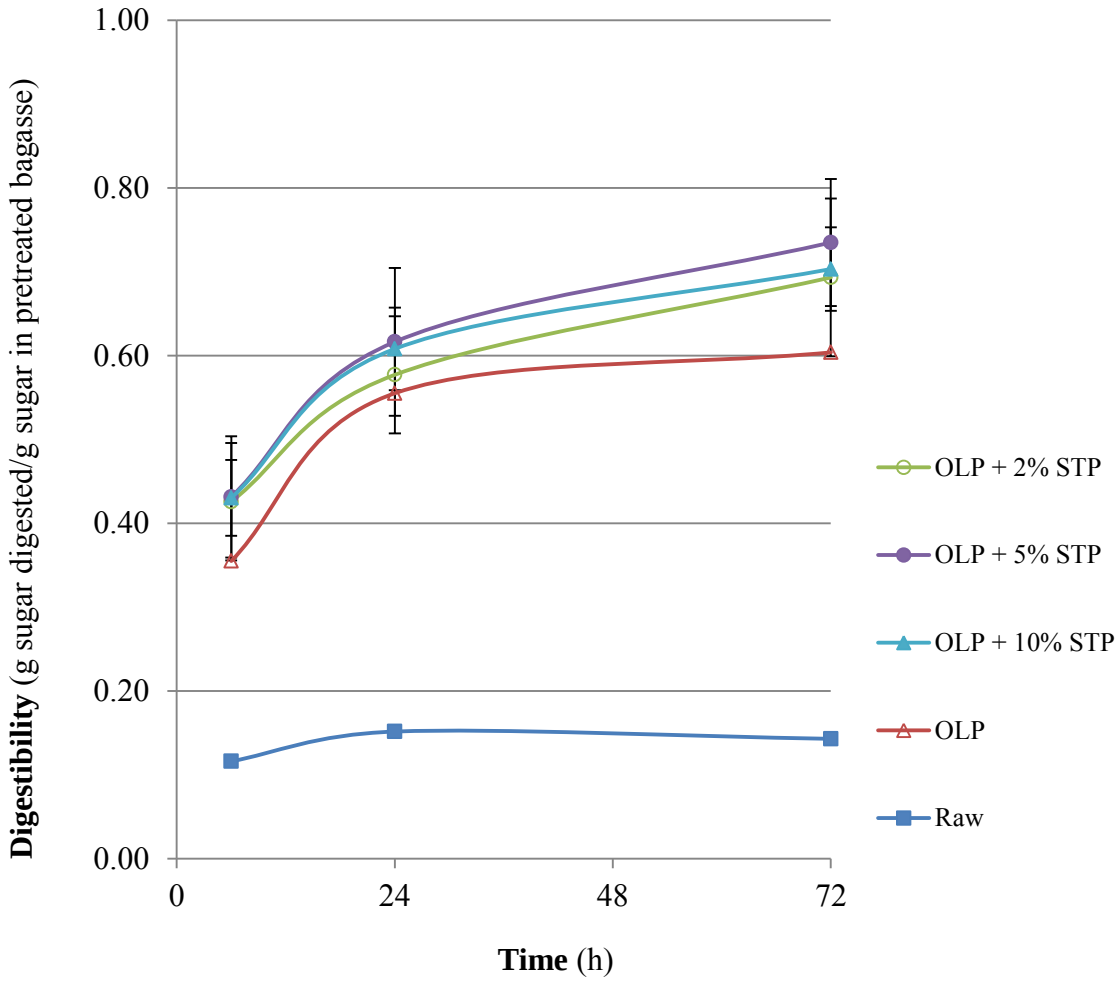

Figure 4-9. Enzymatic digestibility for OLP + STP bagasse at 0.02-, 0.05-, and 0.10-g bagasse/g H_2O solids concentrations. (Cellulase loading = 15 FPU/g glucan in raw bagasse; error bars indicate ± 1 standard deviation.)

Figure 4-10 displays 6-, 24-, 72-h sugar yield for OLP + STP bagasse at 0.02-, 0.05-, and 0.10-g bagasse/g H_2O solids concentrations. At 6 and 24 h, sugar yield for the three OLP + STP samples was not significantly greater than for the OLP sample; however, after 72 h, the sugar yield for the OLP + STP samples was significantly higher. At all time periods, yields for the three OLP + STP samples were insignificantly different.

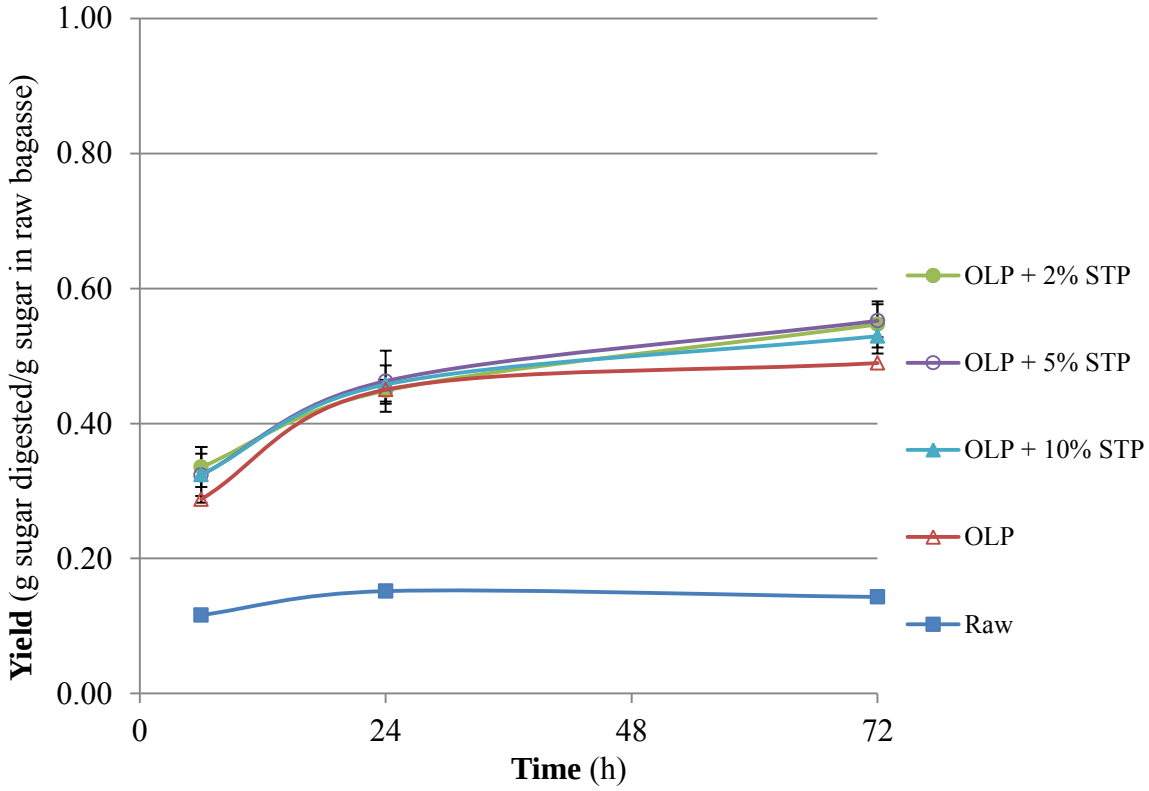

Figure 4-10. Sugar yield for OLP + STP bagasse at 0.02-, 0.05-, and 0.10-g bagasse/g H_2O solids concentrations. (Cellulase loading = 15 FPU/g glucan in raw bagasse; error bars indicate ± 1 standard deviation.)

4.4.2.3 Fill volume

Figure 4-11 displays 6-, 24-, and 72-h enzymatic digestibility for OLP + STP bagasse at 1- and 2-L fill volumes. At 6 h, 1- and 2-L fill volume enzymatic digestibility was significantly greater than for OLP bagasse, and enzymatic digestibility for 2-L fill volume was not significantly different than for 1-L fill volume. Through 24 h, 2-L fill volume enzymatic digestibility was significantly greater than OLP bagasse but insignificantly greater than 1-L fill volume. Enzymatic digestibility for 1-L fill volume was insignificantly greater than OLP bagasse. After 72-h enzymatic hydrolysis, digestibility for 1- and 2-L fill volume was significantly greater than OLP bagasse. Overall, 2-L fill volume enzymatic digestibility was insignificantly greater than enzymatic digestibility for 1-L fill volume samples at 6 h (0.44 and 0.42 g sugar digested/g sugar in pretreated bagasse), 24 h (0.63 and 0.57 g sugar digested/g sugar in pretreatment bagasse), and 72 h (0.73 and 0.69 g sugar digested/g sugar in pretreated bagasse). STP performed on OLP bagasse significantly increased enzymatic digestibility when a 2-L fill volume was used.

Figure 4-12 shows 6-, 24-, and 72-h sugar yield for 1- and 2-L fill volumes. Although the data were more tightly grouped, yield for 1- and 2-L fill volumes was significantly greater than for OLP bagasse at 6 and 72 h. There was almost no difference between OLP + STP data sets.

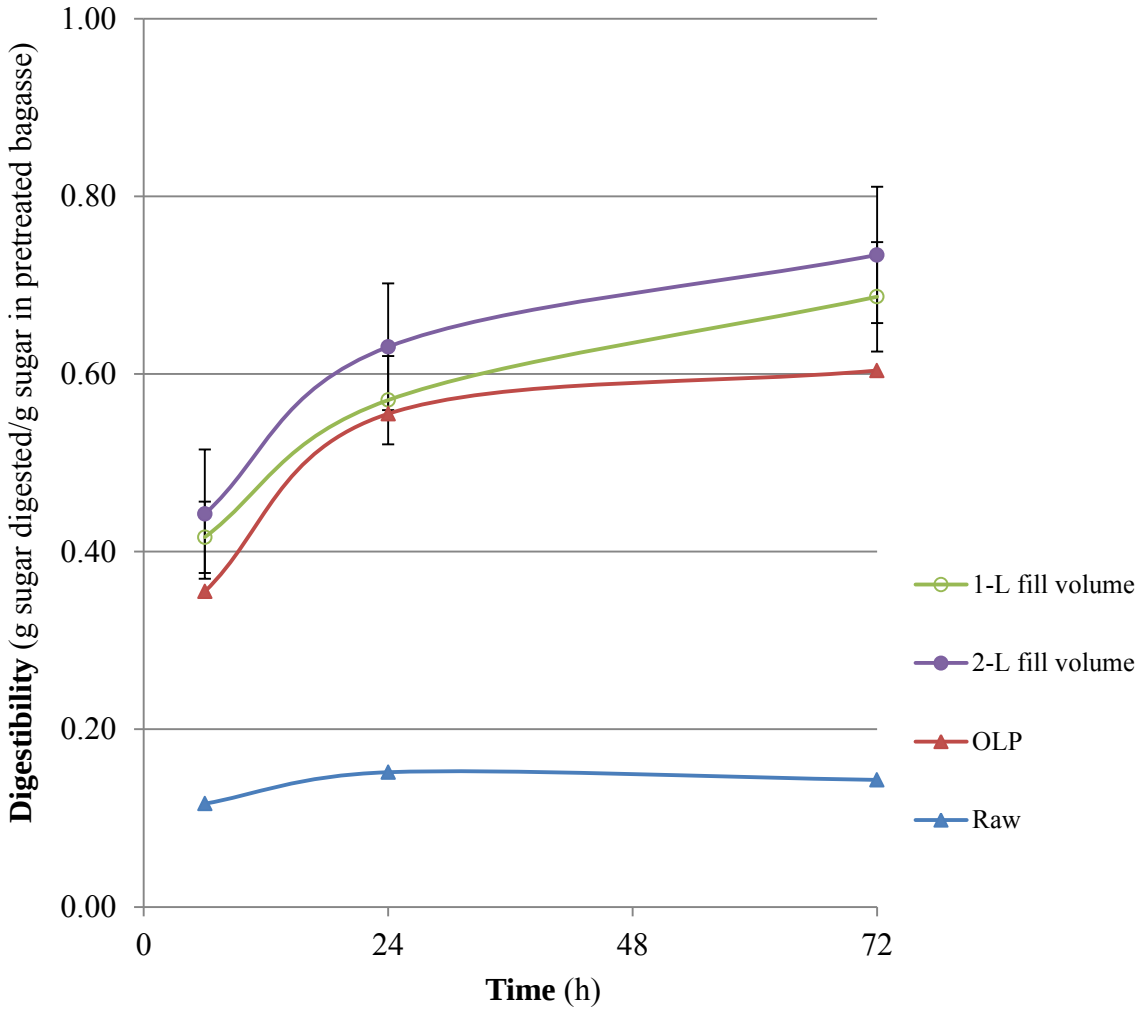

Figure 4-11. Enzymatic digestibility for OLP + STP bagasse at 1- and 2-L fill volumes. (Cellulase loading = 15 FPU/g glucan in raw bagasse; error bars indicate ± 1 standard deviation.)

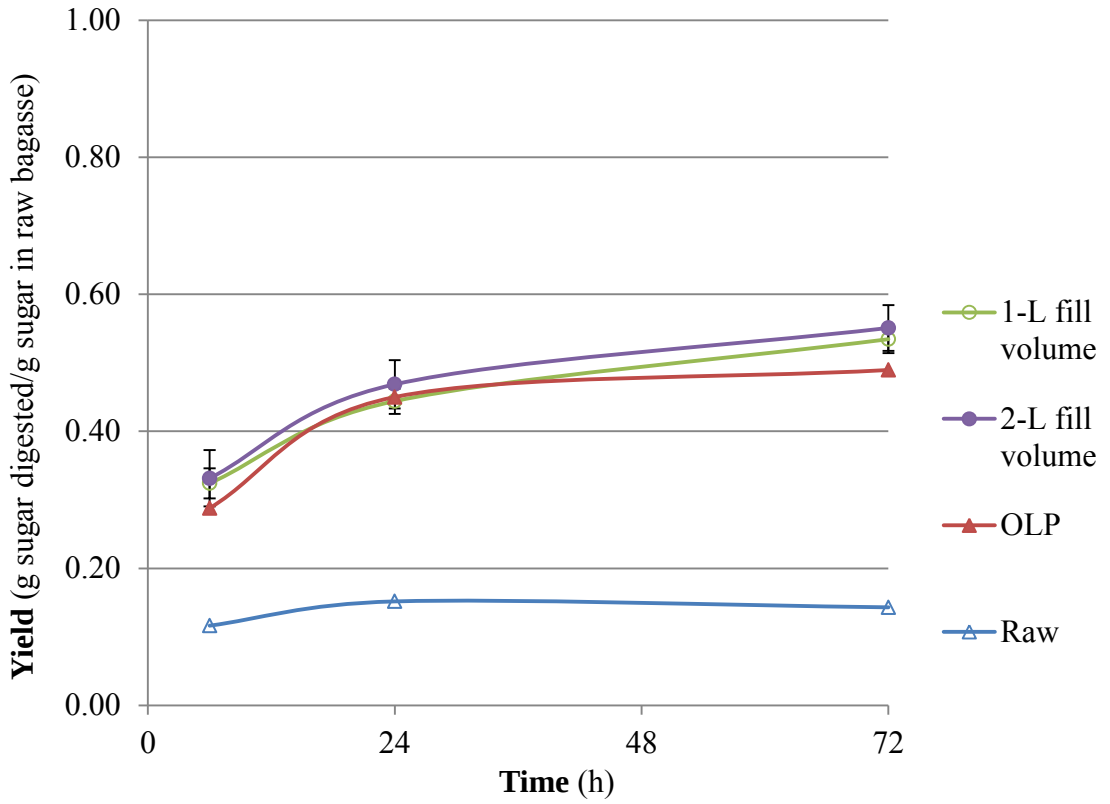

Figure 4-12. Sugar yield for OLP + STP bagasse at 1- and 2-L fill volumes. (Cellulase loading = 15 FPU/g glucan in raw bagasse; error bars indicate ± 1 standard deviation.)

4.4.2.4 Pretreatment order

Figure 4-13 shows 6-, 24-, and 72-h enzymatic digestibility for raw, OLP, STP, OLP + STP, and STP + OLP bagasse. For the STP + OLP sample, STP 11 was used because STP 11 was performed at the nominal conditions. STP decreased enzymatic digestibility compared to raw bagasse; however, STP was successful at increasing enzymatic digestibility on OLP bagasse. For each time period, OLP and STP + OLP bagasse exhibited very similar enzymatic digestibility. The recommended pretreatment order was OLP + STP.

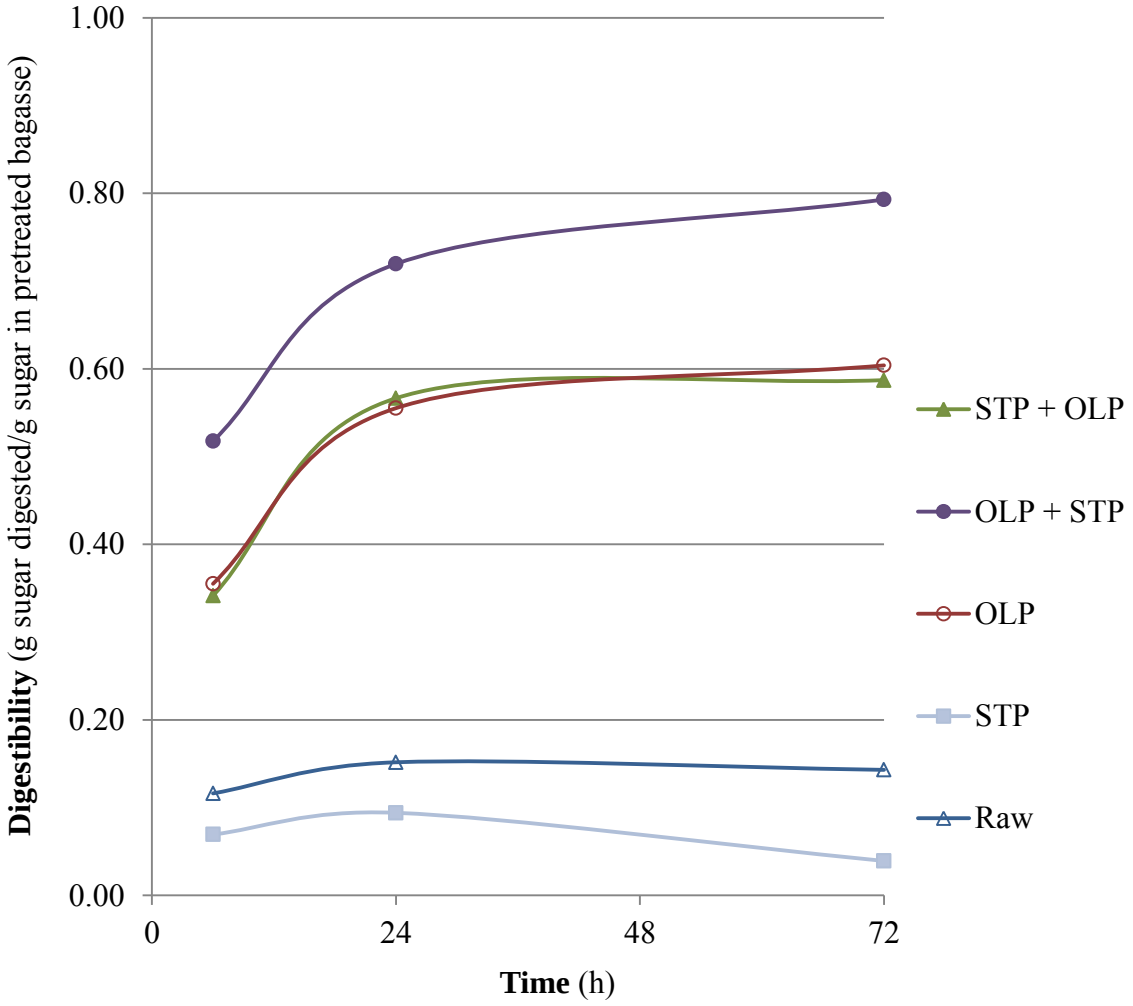

Figure 4-13. Enzymatic digestibility for raw, STP, OLP, OLP + STP, and STP + OLP bagasse. (Cellulase loading = 15 FPU/g glucan in raw bagasse.)

Figure 4-14 shows 6-, 24-, and 72-h sugar yield for each pretreatment order. After 6, 24, and 72 h, yield for the nominal OLP + STP condition (STP 11) was higher than for the other samples (0.38, 0.53, and 0.58 g sugar digested/g sugar in raw bagasse, respectively). Yield for OLP bagasse was slightly better than for STP + OLP bagasse. Yield for STP bagasse was actually lower than for raw bagasse.

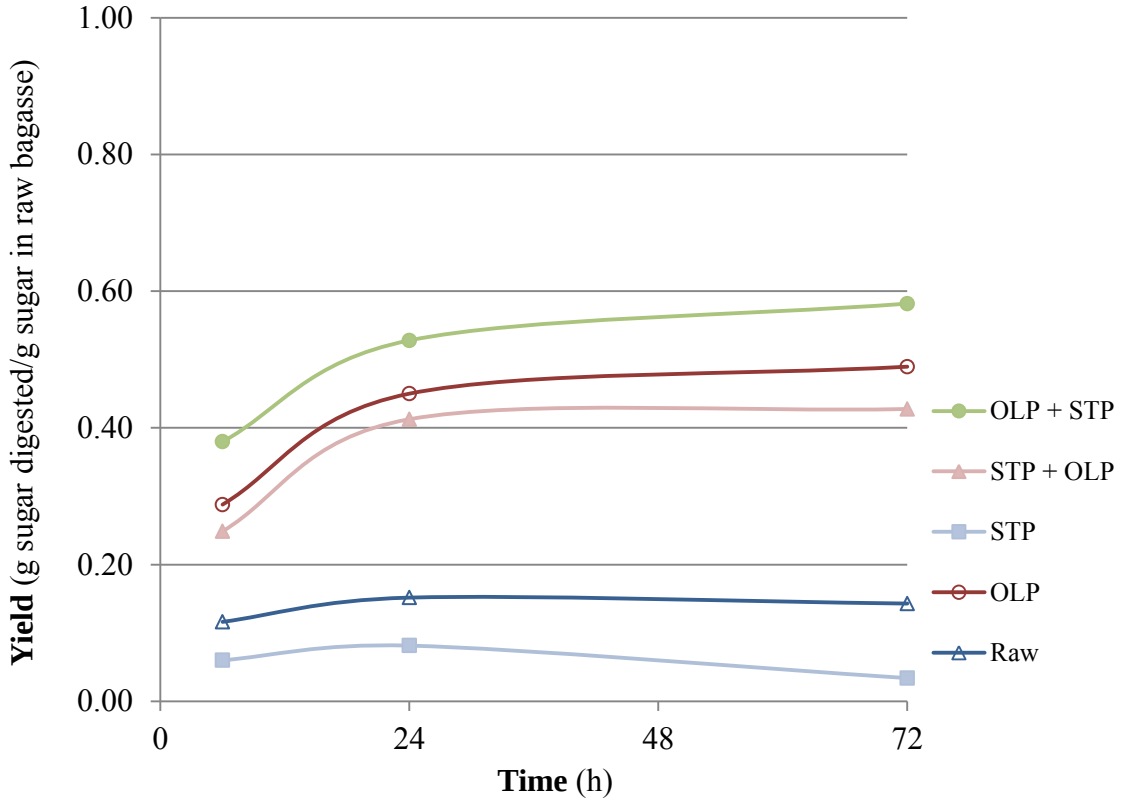

Figure 4-14. Sugar yield for raw, STP, OLP, OLP + STP, and STP + OLP bagasse. (Cellulase loading = 15 FPU/g glucan in raw bagasse.)

4.4.2.5 Shotgun shell type and number of shocks

Figure 4-15 displays 6-, 24-, and 72-h enzymatic digestibility for raw, OLP, and three OLP + STP bagasse samples. Among the OLP + STP samples, one was STP treated using a 00 shotgun shell (nominal is BB) and one was STP treated three times (nominal is one time). For comparison to nominal conditions, STP 11 is also shown. Enzymatic digestibility for each of the three OLP + STP samples was higher than for the OLP sample. After 6-h, enzymatic digestibility for the 00 shell STP was slightly lower than for OLP + STP, but was greater than other samples after 72 h (0.848 g sugar

digested/g sugar in pretreated bagasse). Enzymatic digestibility for three-time STP bagasse was lower than for nominal OLP + STP and 00 shotgun shell OLP + STP bagasse. For each set of STP conditions, STP was effective at improving enzymatic digestibility on OLP bagasse.

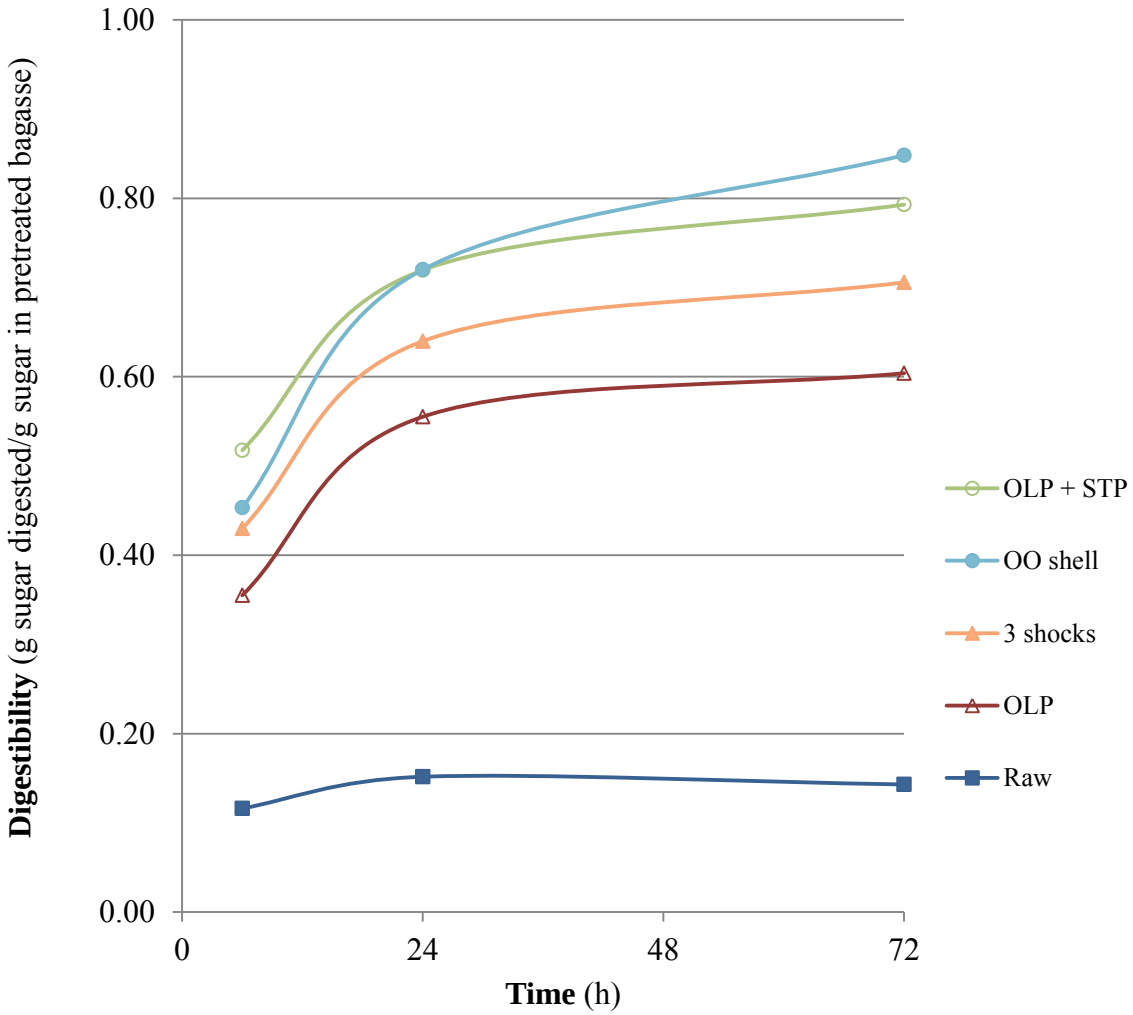

Figure 4-15. Enzymatic digestibility for raw and pretreated bagasse. (Cellulase loading = 15 FPU/g glucan in raw bagasse.)

Figure 4-16 shows 6-, 24-, and 72-h sugar yield for raw, OLP, nominal OLP + STP, OLP + STP (00 shell), and OLP + three-time STP bagasse. At each time period, yield for the nominal OLP + STP (STP 11) sample was greater than for the other samples. Between OLP and nominal OLP + STP bagasse, yields for the 00 shotgun shell and three-shock samples were very close at each time period.

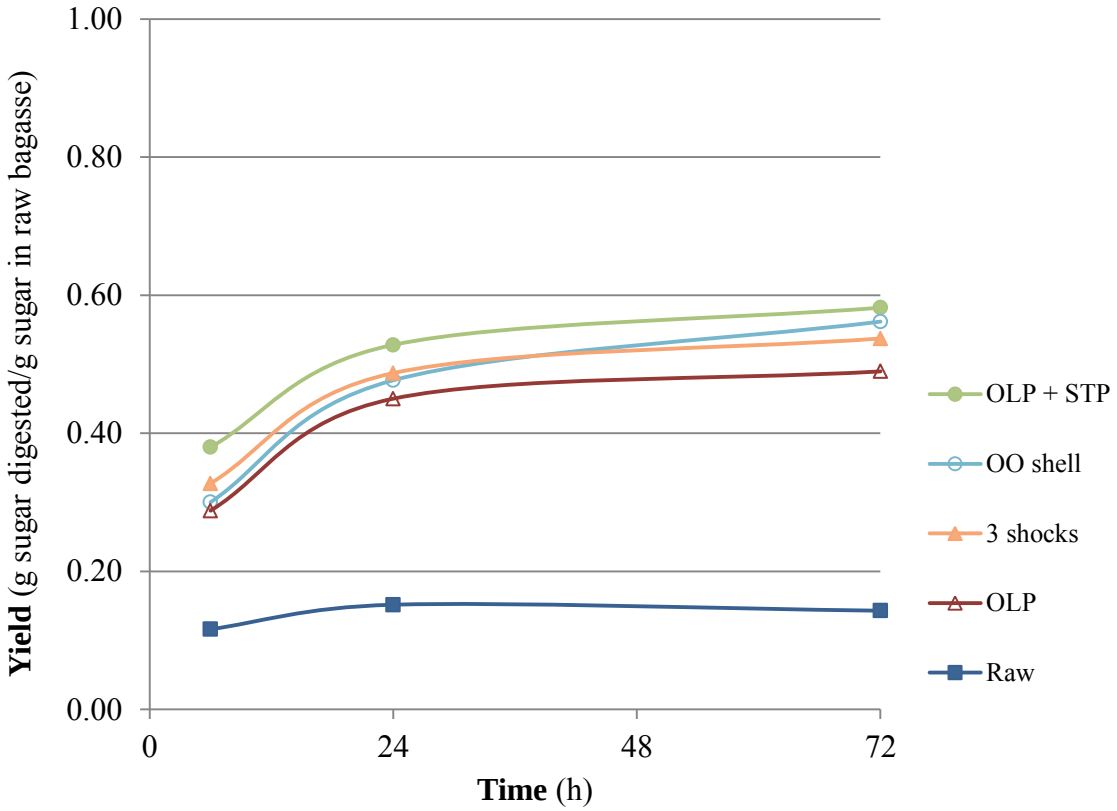

Figure 4-16. Sugar yield for raw and pretreated bagasse. (Cellulase loading = 15 FPU/g glucan in raw bagasse.)

4.4.2.6 72-h enzymatic digestibility

Figure 4-17 shows 72-h enzymatic digestibility for raw, extractives-free bagasse, and STP 1–6. With an enzymatic digestibility less than 0.20 g sugar digested/g sugar in pretreated biomass, the raw sample was much less digestible than all six OLP + STP samples. Enzymatic digestibility was greatest for STP 6 (10% solids concentration, 2-L fill volume) at 0.73 g sugar digested/g sugar in pretreated biomass, and was between 0.60 and 0.70 g sugar digested/g sugar in pretreated biomass for STP 1–5.

Figure 4-18 shows 72-h sugar yield for raw, OLP, and –4/+20 OLP + STP bagasse. Although slightly higher than OLP bagasse (0.49 g sugar digested/g sugar in raw bagasse), yields for OLP + STP bagasse samples were each lower than 0.60 g sugar digested/g sugar in raw bagasse. Yields for pretreated samples were higher than for raw bagasse (0.14 g sugar digested/g sugar in raw bagasse). OLP + STP effectively increased sugar yield by a factor of four.

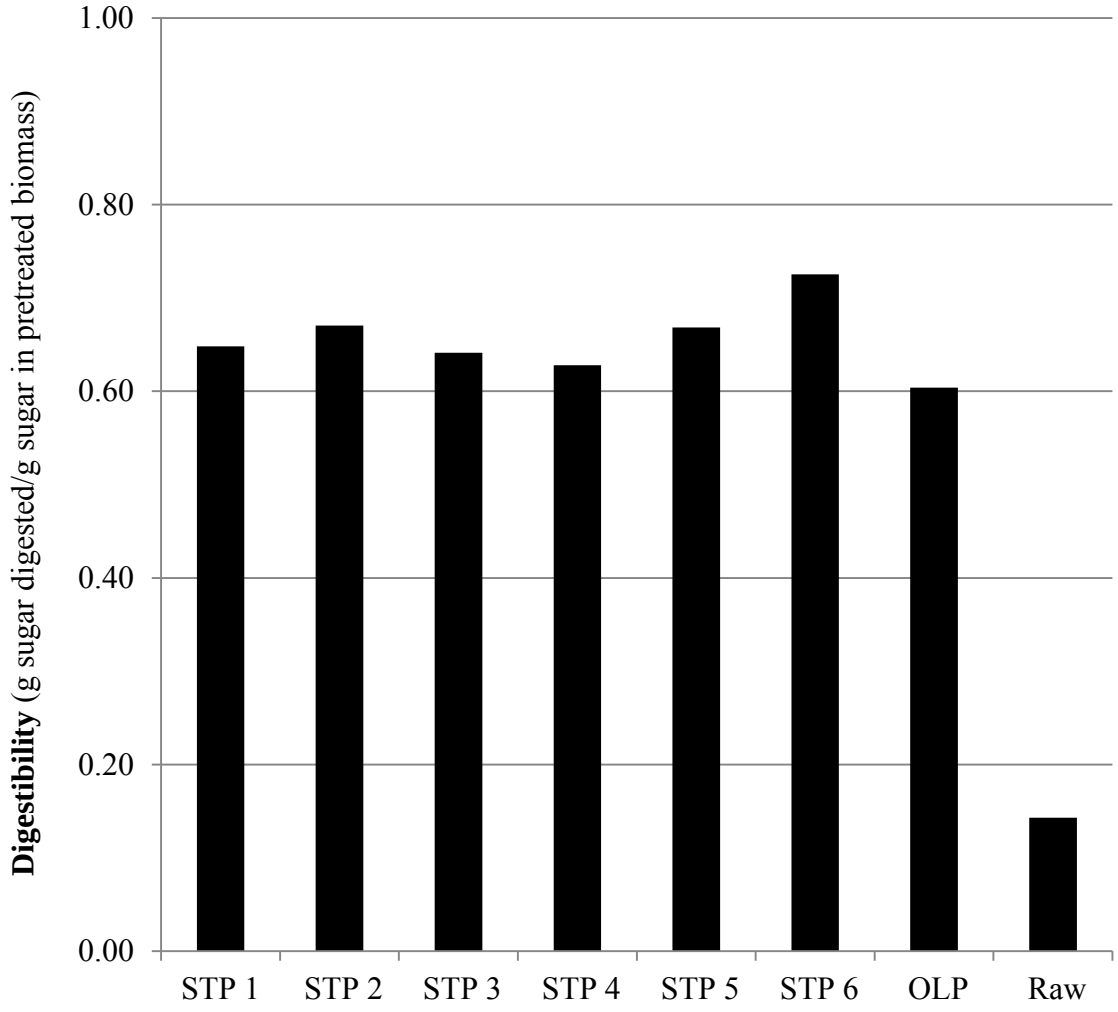

Figure 4-17. 72-h enzymatic digestibility for raw, OLP, and –4/+20 OLP + STP bagasse. (Cellulase loading = 15 FPU/g glucan in raw bagasse.)

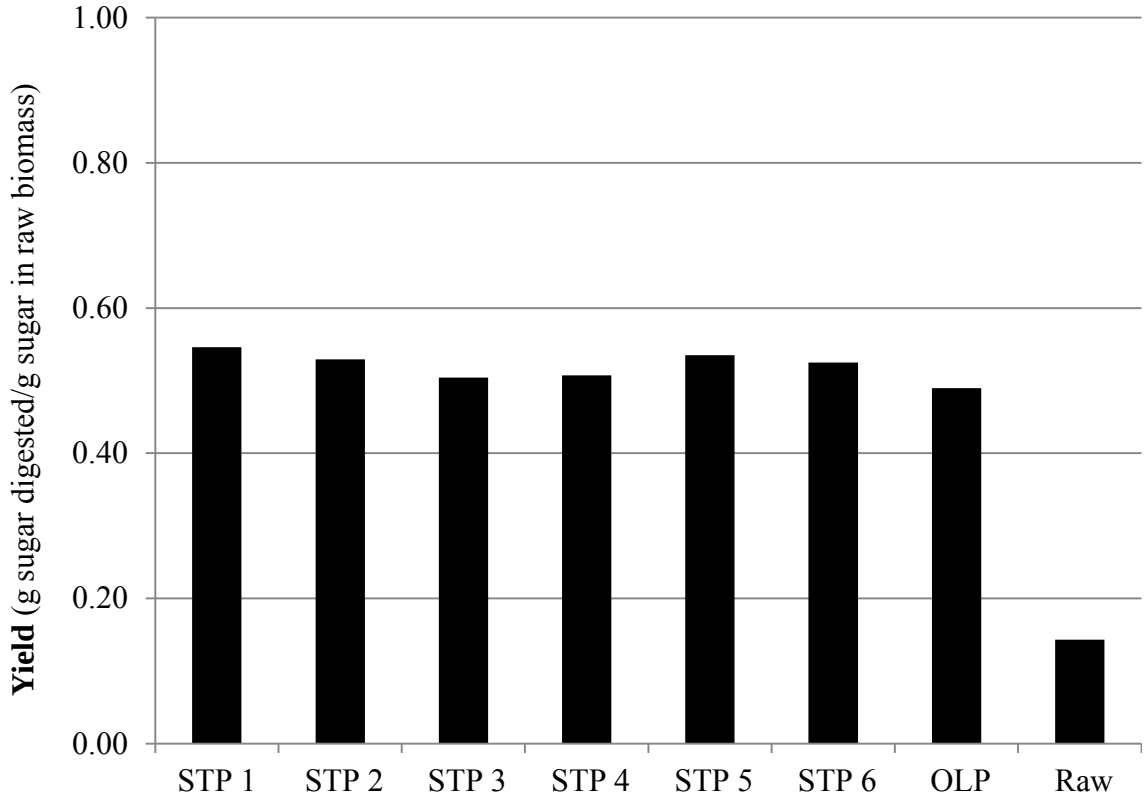

Figure 4-18. 72-h sugar yield for raw, OLP, and –4/+20 bagasse. (Cellulase loading = 15 FPU/g glucan in raw bagasse.)

Figure 4-19 shows 72-h enzymatic digestibility for raw, OLP, and STP 7–12 bagasse. OLP bagasse enzymatic digestibility was slightly lower than for STP 7 and STP 9. STP 10 (2% solids concentration, 1-L fill volume) had the highest enzymatic digestibility at 0.83 g sugar digested/g sugar in pretreated bagasse. Enzymatic digestibility for STP 11 and 12 was between 0.70 and 0.80 g sugar digested/g sugar in pretreated biomass.

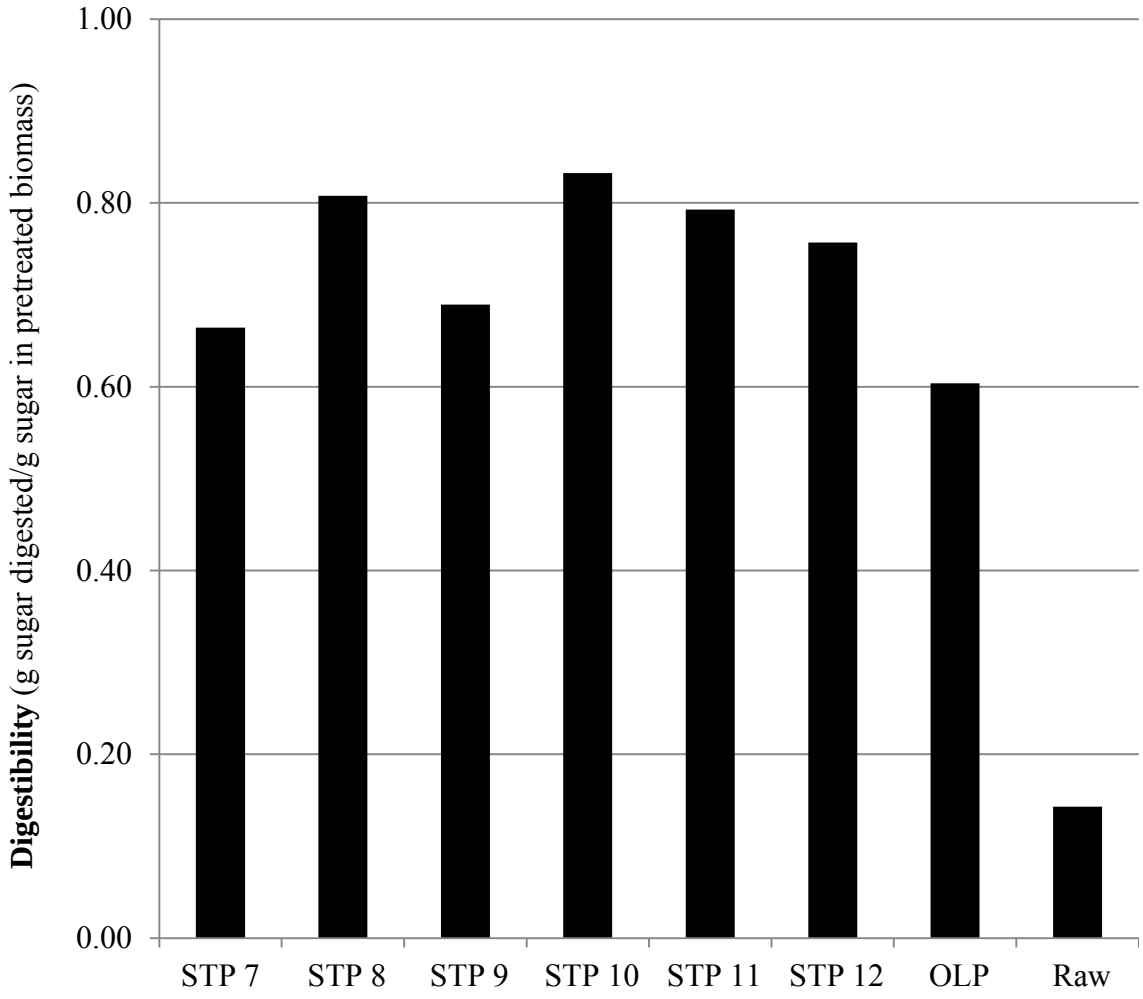

Figure 4-19. 72-h enzymatic digestibility for raw, OLP, and –20/+40 OLP + STP bagasse. (Cellulase loading = 15 FPU/g glucan in raw bagasse.)

Figure 4-20 displays 72-h sugar yield for –20/+40 OLP + STP bagasse. Yield was highest for STP 10 (0.59 g sugar digested/g sugar in raw bagasse), although each was at least 0.54 g sugar digested/g sugar in raw bagasse, slightly greater than for OLP bagasse (0.49 g sugar digested/g sugar in raw bagasse).

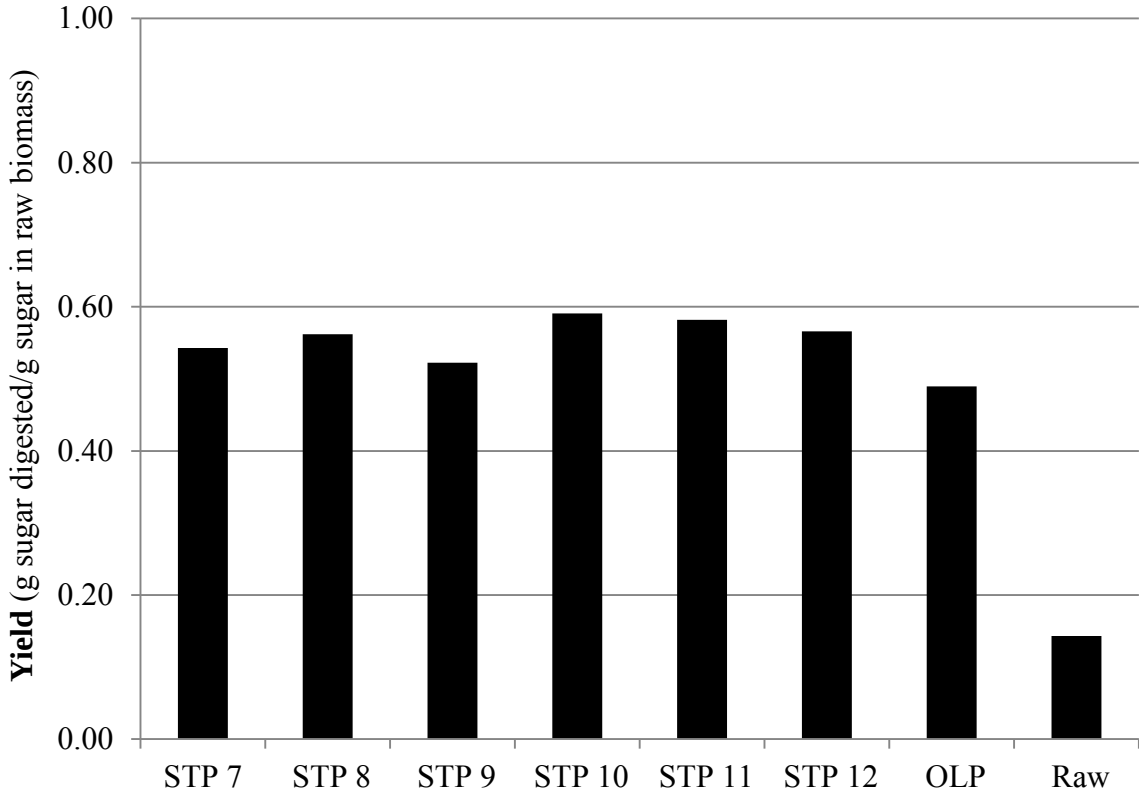

Figure 4-20. 72-h sugar yield for raw, OLP, and –20/+40 OLP + STP bagasse. (Cellulase loading = 15 FPU/g glucan in raw bagasse.)

Figure 4-21 shows 72-h enzymatic digestibility for raw, OLP, STP, STP + OLP, and three OLP + STP bagasse samples. STP bagasse enzymatic digestibility was lower than all other samples studied (0.04 g sugar digested/g sugar in pretreated bagasse). STP + OLP and OLP bagasse samples showed similar enzymatic digestibility around 0.60 g sugar digested/g sugar in pretreated bagasse. Although shocking the OLP bagasse three times showed a slight increase in enzymatic digestibility, OLP bagasse shocked with a 00 shotgun shell was significantly more digestible (0.85 g sugar digested/g sugar in pretreated bagasse).

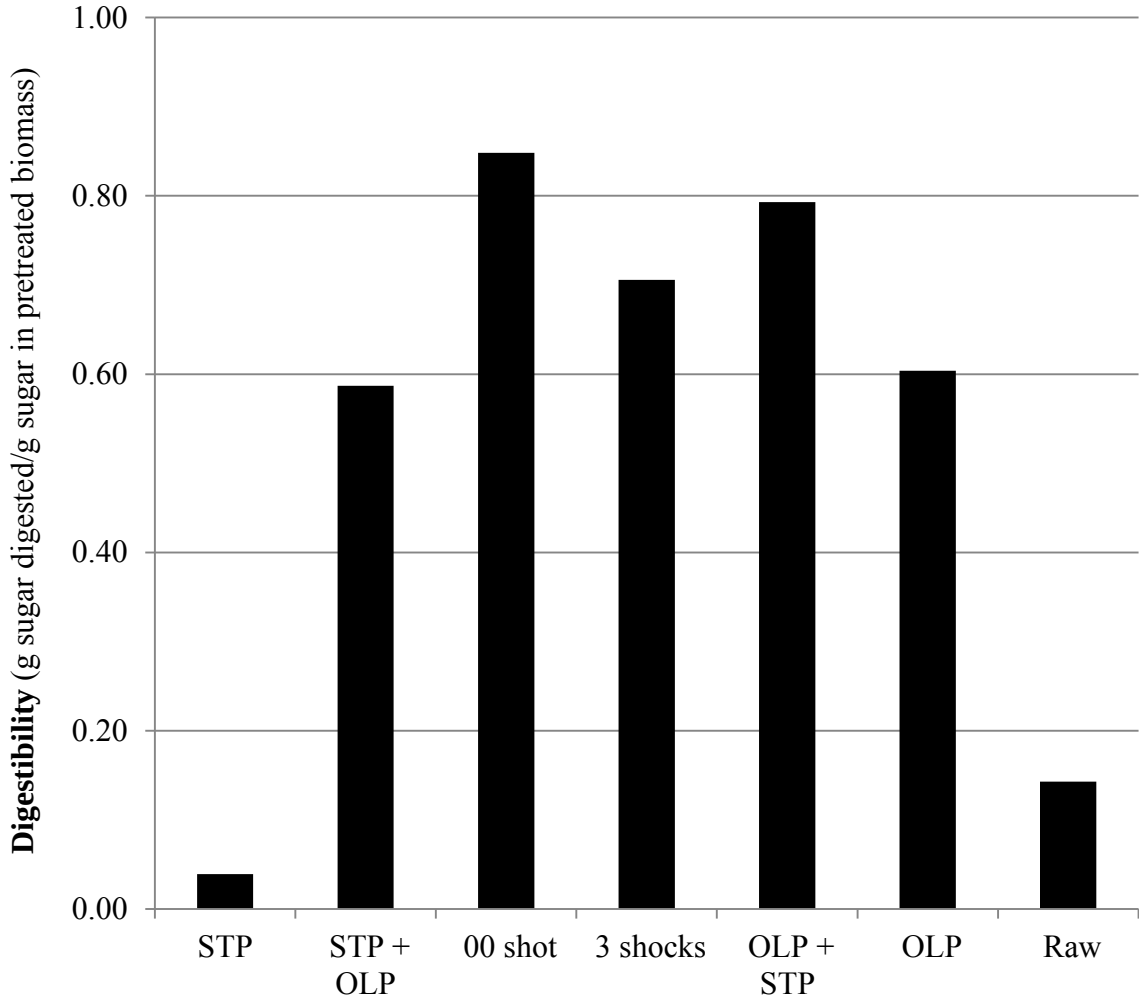

Figure 4-21. 72-h enzymatic digestibility for raw, OLP, STP, STP + OLP, and OLP + STP bagasse. (Cellulase loading = 15 FPU/g glucan in raw bagasse.)

Figure 4-22 displays 72-h sugar yield for raw, STP, OLP, and STP 15–17. Yield for STP was lower than for raw bagasse, and was lower for STP + OLP (0.43 g sugar digested/g sugar in raw bagasse) than for OLP (0.49 g sugar digested/g sugar in raw bagasse). Yields for 00 shotgun shell OLP + STP and OLP + three-time STP were

slightly lower than for the nominal OLP + STP condition, but were higher than for OLP bagasse.

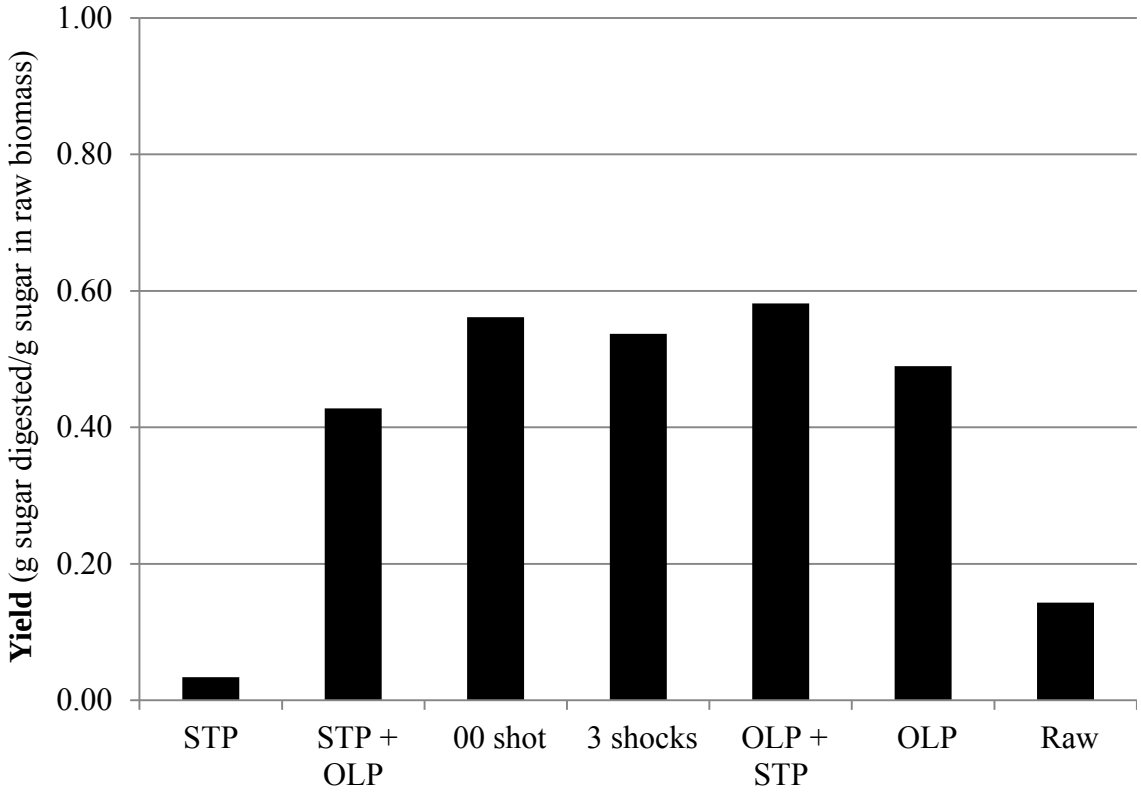

Figure 4-22. 72-h sugar yield for raw, OLP, STP, STP + OLP, and OLP + STP bagasse. (Cellulase loading = 15 FPU/g glucan in raw bagasse.)

4.5 Conclusion

To produce highly digestible sugarcane bagasse, oxidative lime pretreatment and shock tube pretreatment were performed. Material value was determined by enzymatic digestibility and sugar yield. Using a 2-h duration and 6.9-bar O_2, OLP at 130°C produced the most digestible bagasse at the highest yield. STP parameters were then

investigated. Using STP, OLP bagasse was further pretreated. Although specific recommended STP conditions were not found, several trends were discovered. Testing suggested that OLP and STP were more effective on smaller particle-size bagasse, 2-L fill volume, OLP + STP pretreatment order, and 00 shotgun shell. Performing STP multiple times showed a slight increase in enzymatic digestibility as well. STP solids concentration did not affect bagasse enzymatic digestibility.

CHAPTER V

FIXED-BED BATCH FERMENTATION OF PRETREATED BAGASSE

5.1 Introduction

Previous work has demonstrated success in fermenting lime-pretreated bagasse (Domke, 1999; Thanakoses, 2002; Fu, 2007); however, bagasse was pretreated using less effective non-oxidative lime pretreatment. Use of oxidative lime pretreatment (OLP), described by Sierra et al. (2009) and Falls et al. (2011), should improve bagasse fermentation performance compared to past work.

Since 1998, Holtzapple-group fermentations have commonly been performed in 1-L Beckman bottles. Stirring is performed by agitator rods (1/4-in-diameter stainless steel tubes), as described in Section 2.2.5. Liquid-solid separation is performed by centrifugation, as described in Section 2.3. These 1-L bottle fermentations are simply executed but do not simulate industrial-scale pile fermentations, in which liquids pass through a fixed bed of solids. Pile fermentations use gravity percolation for stirring and a passive separation system consisting of a filter below the pile.

Previous research demonstrated that polyester mesh is a satisfactory filter material (Agbogbo, 2005). Liquid selectively permeated through the filter, whereas solids were retained inside the fermentor. However, during long-term fermentation (greater than 200 days), filters became plugged as substrate digested and lost structural integrity. It may be possible to maintain liquid flow through the filter by adding an additional structural support material to the fermentor. If successful, this structural

support retains fine particles throughout the pile and prevents their accumulation at the filter and thus maintains flow. In addition, the structural support material may improve fermentation performance by increasing the amount of solid in contact with liquid and decreasing liquid channeling.

The goals of this work were to:

- determine a suitable structural support material
- investigate the ability of a structural support material to prevent filter plugging
- investigate the ability of a structural support material to improve fermentation performance
- determine the optimal ratio of substrate to structural support
- determine acid yield from OLP + STP bagasse fermentation in a fixed-bed fermentation

5.2 Materials and methods

5.2.1 Fixed-bed fermentor

Fixed-bed fermentors were constructed using PVC pipes and fittings (see Figure 5-1). A 20-in-long section of 2-in-diameter pipe comprised the outer wall of the fermentor. Near the bottom, a filter consisting of polyester fiber placed between two sheets of stainless steel mesh was added to preferentially allow liquids to exit the fermentor. A 14-in-long section of 3-in-diameter pipe was placed around the 2-in-diameter pipe and secured by gluing 3-in × 2-in slip reducers to the pipes at the top and bottom edges of the 3-in-diameter pipe. The annular area between the pipes was used as a hot-water jacket. A 1/4-in-diameter nipple was placed in a threaded hole in each

reducer to allow hot-water circulation. Male adapters were glued to the top and bottom edges of the 2-in-diameter pipe.

A liquid collection area – consisting of an upper ball valve, clear PVC pipe, and lower ball valve – was attached to the bottom of each fermentor. Liquid passing through the polyester filter collected at the bottom of the clear PVC pipe. The clear PVC pipe allowed visual monitoring of the exiting liquid volume. To decrease the minimum liquid volume threshold that could be visually seen through the clear PVC pipe, a PVC insert was placed inside the bottom end of the clear PVC pipe (Figure 5-2). Two modifications were made to the PVC insert: (1) a 1/2-in-diameter drilled hole located at the center allowed liquid to flow through the insert during liquid sampling, and (2) to reduce liquid holdup on the top surface, the top surface was tapered 10 degrees radially toward the center. The PVC insert decreased the minimum visual liquid volume observable in the collection assembly from 140 to 30 mL.

Liquid flow from the fermentor to the liquid collection pipe expanded gas in the fermentor headspace and compressed gas in the liquid collection pipe, prohibiting further liquid flow. To allow gas flow from the liquid collection pipe to the fermentor headspace, a gas bypass was added to the system.

Gas venting and compositional analysis were performed as described in Sections 2.2.6.3 and 2.2.6.2. A gas sampling and relieving port was manufactured from a No. 10-1/2 rubber stopper, cut glass test tube, rubber septum, and aluminum crimp seal in a similar manner to those in Section 2.2.5. The stopper was secured using a 2-in-diameter threaded PVC cap.

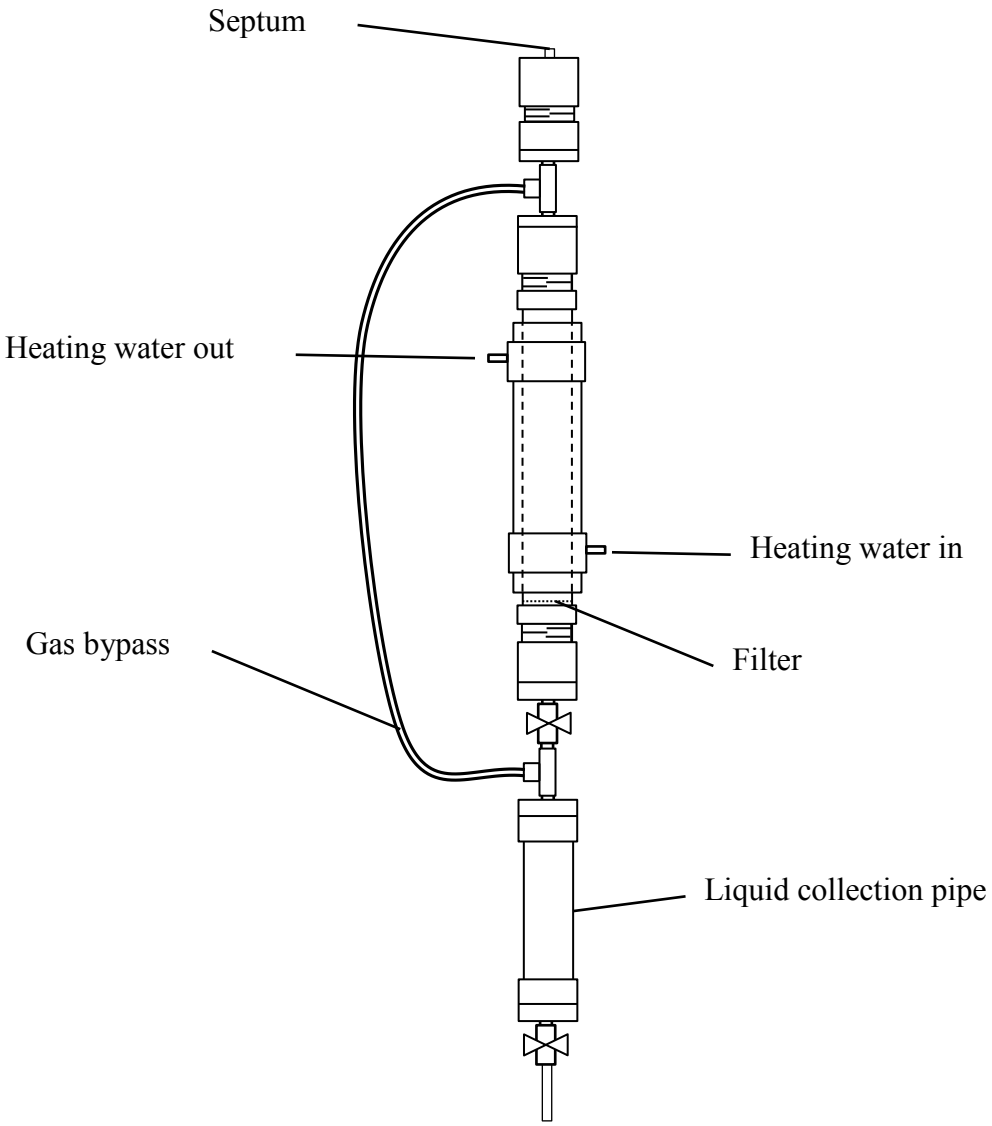

Figure 5-1. Schematic of fixed-bed fermentor.

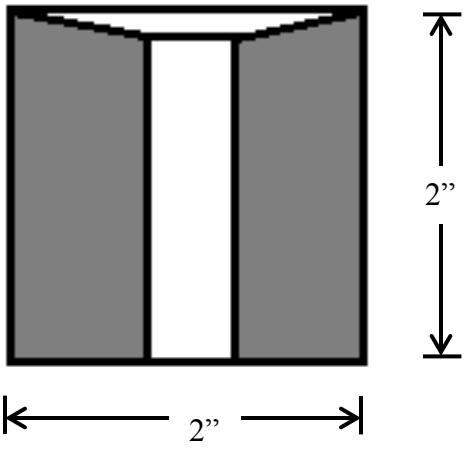

Figure 5-2. PVC insert in liquid collection pipe.

5.2.2 Fermentor heating and setup

Figure 5-3 shows the fermentor and utilities configuration. To maintain fermentor temperature at 40°C, a heating water loop was employed, which consisted of a 20-gal water tank, pump (Teel model 4RJ60), supply manifold, and return manifold. The water loop temperature was controlled using an Omega Engineering CN350 temperature controller connected to two 1500-W immersion heating elements installed to the inner sides of the water tank and a Type K thermocouple. The manifolds were created by threading 1/4-in-diameter NPT barbed fittings into 1-1/4-in-diameter PVC pipes. Flexible plastic tubing was used to connect the manifolds and water jackets. Fermentors were attached to an iron stand using 3-in stainless steel clamps.

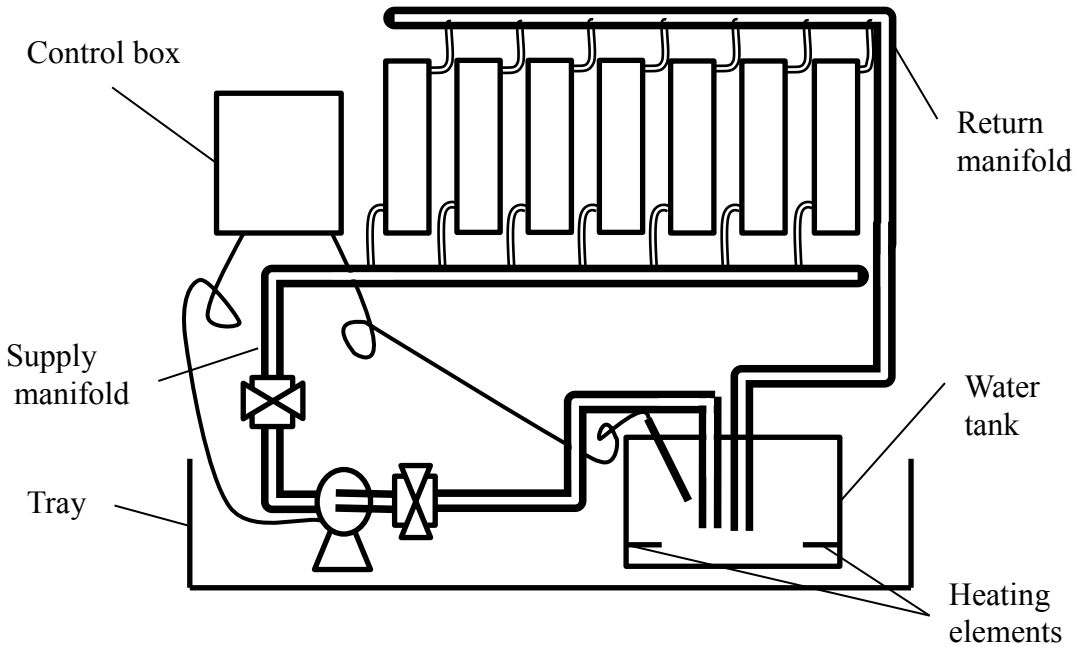

Figure 5-3. Schematic of fixed-bed fermentation setup.

5.2.3 Substrate

The substrate was a mixture of oxidative-lime pretreated (OLP) and shock tube pretreated (STP) sugarcane bagasse (32 g) and dried chicken manure (8 g). Section 4.2 describes OLP and STP in detail. The bagasse was ground to –20/+40 mesh size, pretreated according to the optimal OLP conditions listed in Chapter IV (130 °C, 6.9-bar O_2, 2 h duration), then STP (100 g bagasse, 2 L H_2O per shock, 25°C, BB shotgun shell). Dried chicken manure was obtained from Feathercrest Farms and processed as described in Section 2.2.1.

5.2.4 Inoculum

Galveston inoculum was added to each fermentor initially (50 mL). See Appendix A for detailed collection procedure.

5.2.5 Buffering agents

Sodium bicarbonate and potassium bicarbonate were used in a 50:50 mass ratio. The buffer mixture was added to maintain fermentation pH between 6.8 and 7.2.

5.2.6 Methanogen inhibitor

To prevent methanogen growth, iodoform solution (20 g/L iodoform in ethanol) was added to fermentors (120 μL initially and every 2 days).

5.2.7 Operating conditions

Table 5-1 shows the initial fermentor loading. A total of 15 fermentors were divided into five sets of three fermentors. Each set of fermentors was loaded with a different substrate-to-structural-support ratio. Structural support was washed with DI H_2O and dried at 105°C for 1 h. Bagasse and structural support were mixed in plastic bags according to desired fermentor loading. To saturate the bagasse/structural support mixture with water, it was washed with DI H_2O and filtered through a 140-mesh screen. Other materials (chicken manure, urea, DI H_2O, inoculum, iodoform) were then added to the fermentor.

To allow liquid to flow out of the fermentor and into the liquid collection pipe from the fermentor, the upper ball valve was opened halfway. Opening the valve past halfway resulted in significant liquid flow into the gas sidearm hose, where it became trapped between the compressed gas in the liquid collection pipe and the vacuum in the fermentor.

Each day, liquid was collected from the liquid collection pipe. When pH was below 6.8, buffer mixture was added. Every 2 days, a 3-mL sample was collected and

120 μL iodoform was added. Each time a 3-mL sample was collected, 3 mL of fresh DI H_2O were added to the fermentation liquid. To prevent product inhibition, fermentation liquids with more than 20 g/L acids were diluted by replacing fermentation liquid with DI H_2O.

Table 5-1. Initial fermentation loading.

Item	Set 1	Set 2	Set 3	Set 4	Set 5
Substrate:structural support (g:g)	100:0	95:5	90:10	80:20	70:30
Bagasse (g)	32	32	32	32	32
Chicken manure (g)	8	8	8	8	8
Structural support (g)	0	2.1	4.4	10	17.1
Urea (g)	1	1	1	1	1
DI H_2O (mL)	350	350	350	350	350
Inoculum (mL)	50	50	50	50	50
Iodoform (μL)	120	120	120	120	120

5.2.8 Structural support material

Three potential structural support materials were investigated: stone, pea gravel, and rubber mulch. Each material was expected to be inert during fermentation. Particle size varied greatly for each material. On average, dimensions (cm) for stone, pea gravel, and rubber mulch were 1 × 0.5 × 0.5, 2 × 1.5 × 1.5, and 2.5 × 1 × 1, respectively. Before loading, each candidate material was washed with DI H_2O and dried at 105°C for 1 h. Microcrystalline cellulose (90 μm; Acros Organics) was used to simulate partially digested bagasse.

For each trial, the time required for 300 mL DI H_2O to pass through the fermentor filter was recorded. Table 5-2 details testing parameters. Initially, a predetermined volumetric ratio of cellulose and one of the structural support candidates

were combined with 500 mL DI H_2O in the fermentor. Volumetric ratios of cellulose to structural support included 0:100, 20:80, 40:60, 60:40, 80:20, and 100:0. For Trials 2–6, the initial mass of cellulose was held constant at 30 g. To determine material densities, a 1-L bottle was weighed before and after filling the bottle with material. Stone, rubber mulch, and pea gravel bulk densities were 1.51, 0.47, and 1.59 g/mL, respectively.

Table 5-2. Percolation testing matrix.

Trial	**Cellulose:support** (v:v)	**Cellulose** (g)	**Gravel** (g)	**Rubber mulch** (g)	**Stone** (g)
1A	0:100	---	796	---	---
1B	0:100	---	---	237	---
1C	0:100	---	---	---	754
2A	20:80	30	429	---	---
2B	20:80	30	---	128	---
2C	20:80	30	---	---	407
3A	40:60	30	161	---	---
3B	40:60	30	---	48	---
3C	40:60	30	---	---	153
4A	60:40	30	72	---	---
4B	60:40	30	---	21	---
4C	60:40	30	---	---	68
5A	80:20	30	27	---	---
5B	80:20	30	---	8	---
5C	80:20	30	---	---	25
6	100:0	30	---	---	---
7	0:0	---	---	---	---

A modified fixed-bed fermentor containing a 1/4-in-diameter ball valve in place of the liquid collection pipe was used. Figure 5-4 shows the modified fermentor. Until it was opened at the initiation of a run, the ball valve held up the liquid column. The material exhibiting the least hindrance to percolation, and thus requiring the least amount

of time, was considered optimal because it is best able to allow water flow through the filter.

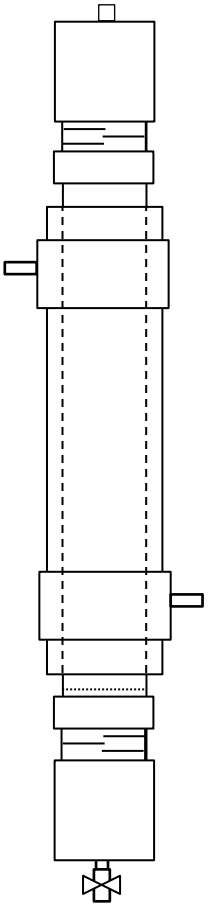

Figure 5-4. Modified fixed-bed fermentor.

5.3 Results and discussion

5.3.1 Determination of optimal structural support material

Figure 5-5 displays percolation times for each volumetric ratio of cellulose to structural support material. When cellulose was not present in the fermentor (ratio

0:100), percolation times were under 10 s for each material. For a ratio of 100:0, percolation time was over 400 s. Rubber mulch outperformed pea gravel and stone in each combined cellulose/support material trial, allowing water to flow through the polyester filter in less time. Most notably, addition of rubber mulch to cellulose decreased percolation time compared to cellulose alone in three of four combined trials. Based on these results, rubber mulch was chosen as the structural support material for subsequent fermentations.

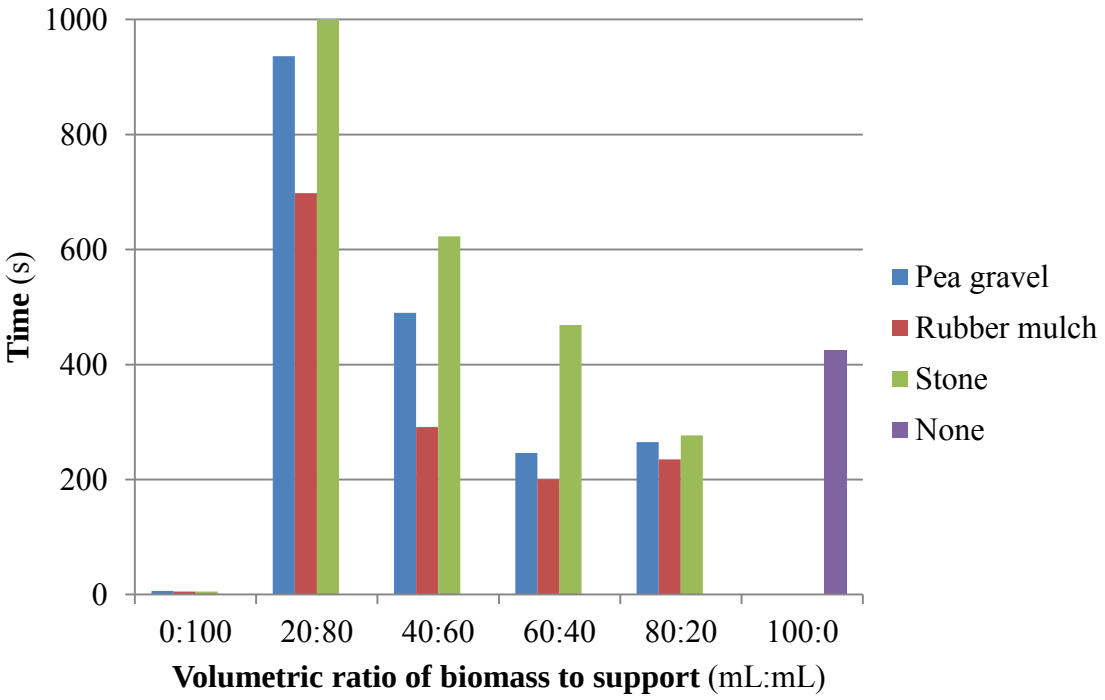

Figure 5-5. Percolation time by volumetric ratio of cellulose to structural support material.

5.3.2 Acid concentration

Figure 5-6 displays average acid concentration for all fermentors. For most sample periods, set acid concentration averages were within 1 standard deviation of each other. After 16 d, acid concentration was around 6 g/L. Throughout the remaining time, acid concentration increased to about 8 g/L. Because of low acid concentration, fermentation operation was changed on Day 34. Fermentation liquids were retained in fermentors for approximately 23 h, in contrast to immediate release during the first 34 days.

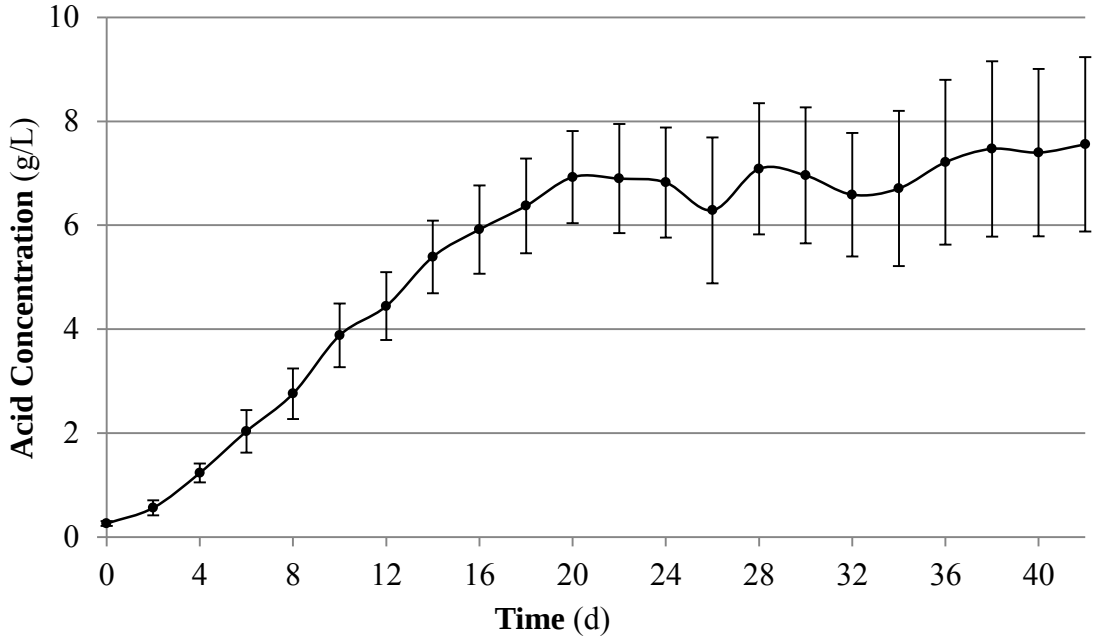

Figure 5-6. Average acid concentration. (Error bars indicate ± 1 standard deviation.)

5.3.3 Percolation

Figure 5-7 shows set-averaged percolation volumes. Nominal initial liquid volume available for percolation was 400 mL. Filtering with a mesh screen was unable to remove all supersaturated DI H_2O added to substrate prior to loading. For all sets, percolation volumes were greater than 400 mL throughout a majority of the time. Volumes remained fairly constant throughout the 34-d period; however, variation within each set increased with time. After operation change on Day 34, percolation volumes decreased slightly. Final set standard deviations ranged from 50 to 120 mL. Constant percolation volume and low acid concentration suggest that bagasse was not greatly digested.

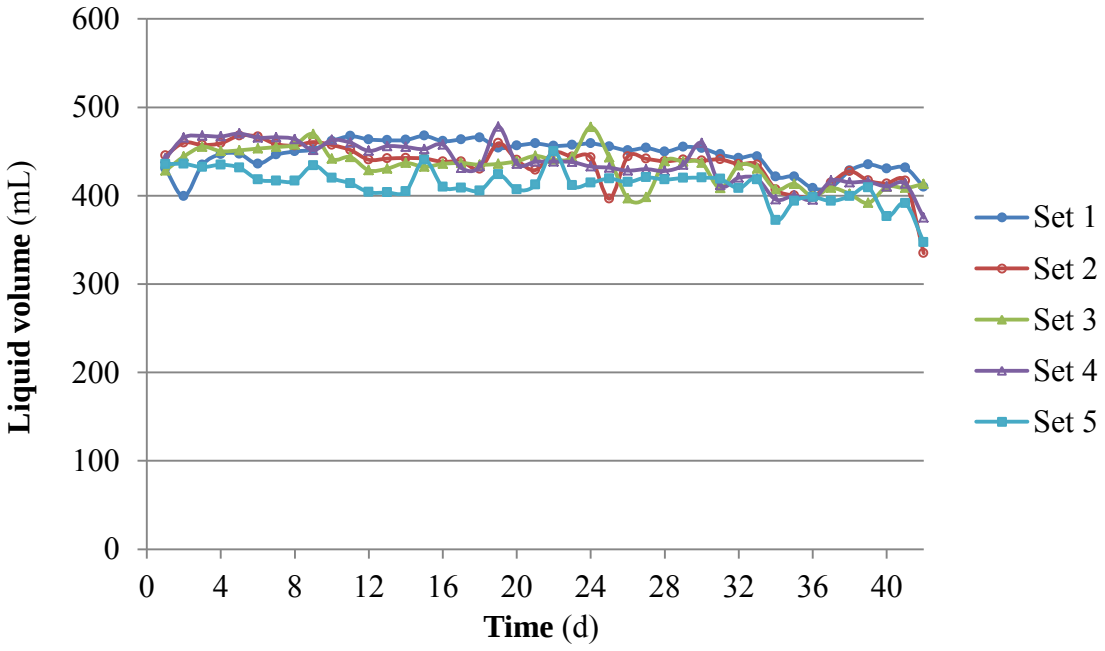

Figure 5-7. Set-averaged percolation volume. (Error bars indicate ± 1 standard deviation.)

5.3.4 Fermentation pH

Figure 5-8 shows average fermentation pH for all fermentors. Target pH was 6.8–7.2. Approximately 0.5 g buffer mixture was added to fermentors when pH was less than 6.8. Target pH was maintained successfully for Days 1–14, before pH increased to more than 7.2 over the next 22 days. The dramatic pH increase from Day 15 to Day 16 corresponded to buffer addition in all fermentors on Day 15. From Day 16 to Day 34, pH was poorly controlled, remaining greater than 7.2. Poor pH control was a result of using basic buffers, and likely hindered fermentation performance. Once pH was outside target range, fermentation acid concentration (Figure 5-6) and yield (Figure 5-9) stopped increasing. By increasing solid-liquid contact time, the operation change on Day 34 effectively decreased pH to the target range on Day 37.

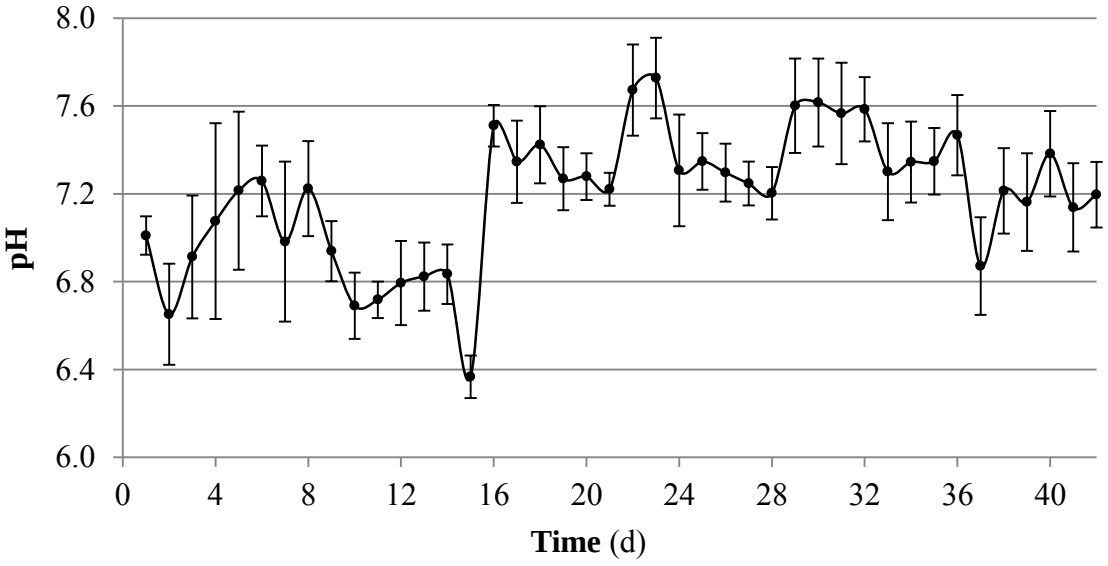

Figure 5-8. Average fermentation pH. (Error bars are ± 1 standard deviations.)

5.3.5 Exit yield

Exit yield is defined in Equation 2-11. Over the first 18 days, Y_E steadily increased to about 0.1 g acids/g $NAVS_{in}$. Afterwards, set averages were nearly constant. The highest set-averaged exit yield was 0.13 g acid/g $NAVS_{in}$ for Set 3.

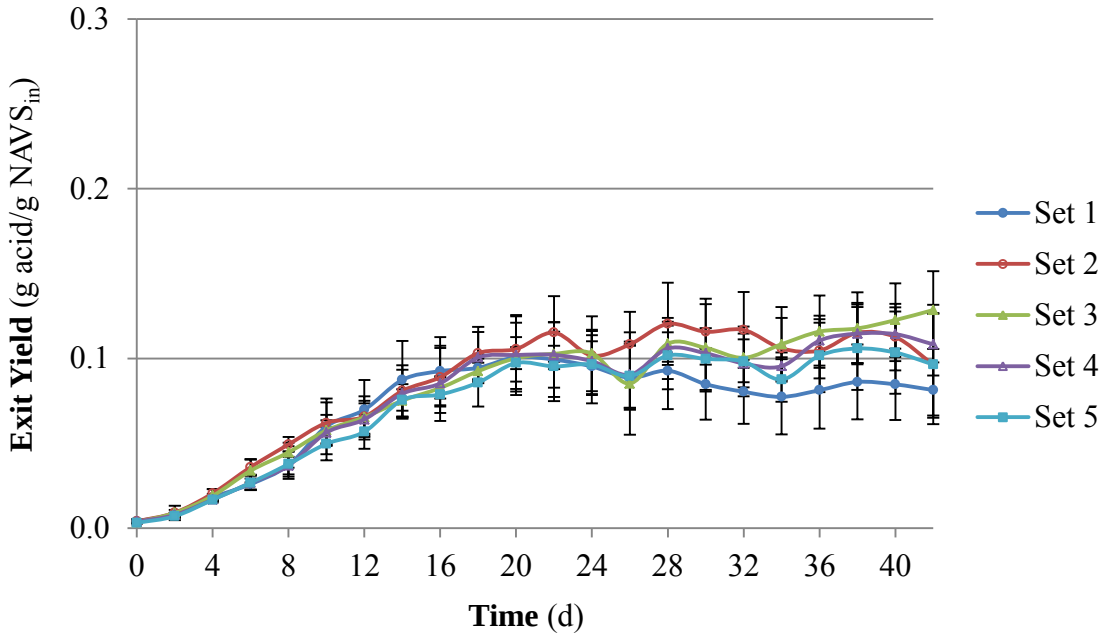

Figure 5-9. Set-averaged exit yield. (Error bars are ± 1 standard deviations.)

5.4 Conclusion

During a 42-day batch fermentation, pretreated (OLP and STP) bagasse and chicken manure were fermented in fixed-bed fermentors. During the first 34 days, fermentation liquids percolated through the solids, were collected, analyzed, and returned to the fermentor. After 34 days, liquids were kept in the fermentors for 23 h per day to provide more solid-liquid contact time. To prevent fermentor filter plugging, sets

of three fermentors were loaded with substrate and varying amounts of rubber mulch. Addition of rubber mulch with microcrystalline cellulose decreased water percolation time through the fermentor filter between 40:60 and 80:20 mL biomass:mL support. Filter plugging leading to decreased percolation volume was not observed, although it was reported in previous work with similar fermentors. Longer-term batch fermentation with more biomass degradation would be expected to lead to increased risk of filter plugging. Because data were tightly grouped across fermentor sets, addition of rubber mulch was not found to significantly impact fermentation metrics. Acid concentration only reached 8 g/L after 42 d because of poor pH control and short solid-liquid contact time. The maximum set-averaged exit yield was 0.13 g acid/g $NAVS_{in}$. In future experiments, contact time should be increased to increase productivity, with careful consideration given to allow adequate percolation time without sacrificing data collection frequency.

CHAPTER VI

DEVELOPMENT OF HIGHLY DIGESTIBLE ANIMAL FEED FROM CORN STOVER

6.1 Introduction

For ruminants, it is possible to displace corn with lignocellulose, the most abundant organic material on earth (Rajarathnam et al., 1989). Unfortunately, because of its structural features, lignocellulose is recalcitrant to digestion and requires pretreatment to increase digestibility. Although many structural features influence lignocellulose digestibility, lignin content, hemicellulose acetyl content, and cellulose crystallinity are among the most important. Previous research has shown that lime pretreatment significantly reduces lignin content and completely removes acetyl groups from hemicellulose (Chang and Holtzapple, 2000). Physical pretreatments (e.g., ball milling) are highly effective at lowering cellulose crystallinity (Bertran & Dale, 1985; Puri, 1984). Furthermore, combining lime pretreatment with mechanical pretreatment dramatically improves enzymatic digestibility (Falls & Holtzapple, 2011).

Two potential sources of lignocellulose are energy crops and agricultural residues. In the United States, corn stover (*Zea mays*) is the most abundant agricultural residue with an availability of approximately 80 million dry tons per year (Kadam & McMillan, 2003).

The purpose of this work was to generate highly digestible corn stover to supplement or replace corn grain as ruminant animal feed. To accomplish this, a

combination of oxidative lime pretreatment (OLP) and shock tube pretreatment (STP) was employed to render corn stover more digestible. To determine the nutritive value of the generated feed, composition and *in vitro* digestibility were determined by university and commercial laboratories.

6.2 Materials and methods

6.2.1 Corn stover feedstock

Corn stover was provided by the National Renewable Energy Laboratory and was dried to uniform moisture content (<10%) and milled to pass through a 1/4-in round screen. To wash extractives out of the corn stover, DI H_2O was used at a ratio of 10 mL DI H_2O per mL corn stover. Corn stover and DI H_2O were mixed on a rolling bed apparatus for 1 h before centrifugation; the solids were subsequently dried. The supernatant was concentrated using rotary evaporation, and then freeze-dried to a powder using a Labconco Lyph-Lock 6-L freeze dryer system (Model 77530, Labconco Corporation, Kansas City, MO). Cumberland Valley Analytical Services, Inc. (CVAS; Hagerstown, MD) performed the compositional analysis displayed in Table 6-1. Samples were analyzed for dry matter (DM) (National Forage Testing Association recommendations, 2002), ash (AOAC, 2000; method 942.05), CP (AOAC, 2000; method 990.03), lignin (Goering and Van Soest, 1970), crude fat (AOAC, 2006; method 2003.05), acid detergent fiber (ADF; AOAC, 2000; method 1973.18), NDF (Van Soest, et al., 1991), and NFC (Dubois, et al., 1956). Cracked corn grain and alfalfa were also analyzed for comparison. CVAS performed compositional analysis on these materials as well, which is displayed in Table 6-1.

Table 6-1. Raw feedstock compositions.

Feedstock	MC	Ash (%DM)	CP (%DM)	ADF (%DM)	NDF (%DM)	NFC (%DM)	Lignin (%DM)	Fat (%DM)
Corn stover	9.5	7.9	6.5	44.5	71.2	15.5	9.6	0.9
Corn grain	14.2	1.3	8.5	4.7	11.2	75.6	2.3	3.9
Alfalfa	7.4	9.4	15.0	35.6	44.6	30.9	8.7	1.9

Analysis provided by Cumberland Valley Analytical Services, Inc.

6.2.2 Pretreatment methods

6.2.2.1 OLP

As described in Section 4.2.1, corn stover was pretreated using OLP. The pretreatment vessel was a 20-L stainless steel batch reactor (Figure 6-1). Corn stover (500 g), excess calcium hydroxide (250 g), and DI H_2O (7.5 L) were loaded into the reactor. The reactor temperature, oxygen pressure and reaction time were 110°C, 6.9 bar, and 3 h, respectively. When the reaction was complete and the reactor had cooled down, the pretreated slurry was removed and neutralized to pH 4 using 5-N HCl. To isolate pretreated solids, the slurry was then vacuum filtered. To wash out residual lime, the pretreated solids were washed with DI H_2O a minimum of three times, until the pH of the collected wash was equal to that of fresh DI H_2O. Appendices F and G provide detailed operating procedures for the 20-L OLP reaction setup and subsequent neutralization, washing, and drying.

Figure 6-1. 20-L OLP reactor.

6.2.2.2 STP

To further increase corn stover digestibility, shock tube pretreatment was then employed. Section 4.2.2 and Appendix H describe the shock tube apparatus and operating procedure.

6.2.3 TDN

The value of each prepared sample as an animal feed was estimated by total digestible nutrients (TDN). TDN was calculated by CVAS using the Weiss model (Weiss et al., 1992), which calculates TDN based on true digestibility coefficients for available soluble carbohydrates, proteins, fatty acids, and fiber.

The Weiss equation is:

$$TDN_w = 0.98(100 - NDFn - CP - ash - EE + IADFIP) + (dCP)(CP) +$$

$$2.25(EE - 1) + 0.75(NDFn - lignin)\left[1 - \left(\frac{lignin}{NDFn}\right)^{\frac{2}{3}}\right] - 7 \quad (6\text{-}1)$$

$$dCP = (CP)e^{[-0.012ADFIP]} \quad (6\text{-}2)$$

$$NDFn = NDF - NDFIP + IADFIP \quad (6\text{-}3)$$

where:

EE = ether extract

ADFIP = average daily feed intake protein

IADFIP = indigestible ADFIP (0.7 × ADFIP for forages)

dCP = digestibility of CP

NDFn = NDF adjusted for nitrogen

NDFIP = NDF-insoluble protein

All values are expressed as % DM.

Texas A&M University Animal Science Department also calculated an adjusted TDN based on measured 48-h neutral detergent fiber digestibility (NDFD48), and used the following equations (Tedeschi et al., 2009):

$$TDN_N = 0.98[100 - (NDF - NDIN) - CP - EE - Ash + dCP + dEE + dNDF - 7] \quad (6\text{-}4)$$

$$dCP = \left[1 - 0.004\left(\frac{(ADIN)(CP)}{100}\right)\right] CP \quad (6\text{-}5)$$

$$dEE = 2.25(EE - 1) \quad (6\text{-}6)$$

$$\mathrm{dNDF} = (\mathrm{NDFD48})(\mathrm{NDF} - \mathrm{NDIN}) \quad (6\text{-}7)$$

where:

NDIN = neutral detergent insoluble nitrogen

dEE = digestible EE

dNDF is ruminal and intestinal digestible NDF

ADIN = acid detergent insoluble nitrogen (% of CP)

All values, except ADIN, are expressed as % DM.

6.2.4 In vitro anaerobic fermentation and gas production

Texas A&M University Animal Science Department analyzed corn stover *in vitro* anaerobic fermentation using the gas production method described previously (Tedeschi et al., 2009). The *in vitro* fermentation chamber included an incubator with a multi-plate stirrer, pressure sensors attached to incubation flasks (125-mL Wheaton bottles), an analog-to-digital convertor device, and a PC-compatible computer provided with appropriate software (Pico Technology, Eaton Socon, Cambridgeshire, UK). The pressure inside each flask was automatically recorded every 5 min for 48 h (2,880 data points). Each incubation flask was loaded with feed sample (200 mg), boiled distilled water that had been cooled to room temperature (2 mL), cysteine hydrochloride (14 mL), and filtered mixed ruminant bacteria inoculum (4 mL). Pressure recording was initiated once the fermentation chamber reached the fermentation temperature (39°C). Fermentation pH was maintained between 6.8 and 6.9. Once fermentation was complete, 40 mL neutral detergent solution was added to each bottle, the bottles were

crimp sealed, and placed in an autoclave for 60 min at 105°C. Undigested fiber was filtered using a Whatman 54 filter paper, and NDF was determined gravimetrically.

6.3 Experimental design

Five corn stover samples and two control samples (corn grain and alfalfa) were analyzed: (1) untreated corn stover, (2) OLP corn stover, (3) STP corn stover, (4) OLP + STP, and (5) STP + OLP corn stover. CVAS analyzed compositional differences, estimated TDN, and measured 30-h *in vitro* NDF digestibility (NDFD). Texas A&M University Animal Science Department measured 48-h *in vitro* NDFD and gas production resulting from anaerobic fermentation.

6.4 Results and discussion

Compositional analysis and digestibility results are given on an organic matter (OM) basis.

6.4.1 Compositional analysis

Compositional analysis was performed to determine changes in composition from pretreatment. Table 6-2 displays compositional analysis of control samples, untreated and treated corn stover, and corn stover extractives. Corn grain had a significantly higher NFC content (76.6%) than both alfalfa (34.1%) and raw corn stover (16.8%), which is why corn grain is widely used in ruminant diets. OLP had a negligible effect on NFC (16.0% compared to 16.8%). However, STP significantly reduced NFC (6.9%). When combined, the order of pretreatment had little effect on NFC content (10.4%). The effect of STP on NFC is not well understood, and needs to be further explored.

Raw corn stover had significantly higher NDF (77.3%) than alfalfa (49.2%) and corn grain (11.4%). The primary hurdle of using lignocellulose in high-quality ruminant feeds is overcoming the high NDF content, which is normally highly indigestible. Both pretreatment processes significantly increased NDF. OLP alone increased NDF to 81.9%, and STP alone increased NDF to 88.1%. Similar to NFC, when combined, the order of pretreatments had little effect on NDF changes. OLP + STP had similar NDF (87.6%) to STP + OLP (87.1%).

The CP content of raw corn stover (7.1%) was only slightly lower than corn grain (8.6%), but considerably lower than alfalfa (16.6%). A significant drawback to using OLP to generate animal feed is the unavoidable degradation of protein. To some extent, protein can be protected by prewashing the corn stover to recover protein prior to OLP. OLP reduced corn stover CP to 3.2%, whereas STP had a negligible effect (6.6%). When combined, OLP + STP and STP + OLP had CP contents of 4.1% and 3.1%, respectively. If OLP is used to produce animal feed, it will be necessary to supplement it with a high-protein source, such as alfalfa, soybean meal, distillers' grains, or solubilized protein (Coward-Kelly et al., 2006).

Neither OLP nor STP significantly affected the mineral composition of corn stover. Slight increases of calcium were observed, particularly with OLP alone (2.9% DM), indicating that extensive washing was unable to fully remove all unreacted calcium ions. OLP also removed the majority of potassium, only leaving trace amounts. Corn stover extractives contained significant levels of calcium (3.3% DM) and potassium (11.7%).

Table 6-2. Compositional analysis of corn grain, alfalfa, and corn stover samples.

Sample	Ash (%DM)	CP (%OM)	ADF (%OM)	NDF (%OM)	NFC (%OM)	Lignin (%OM)	Fat (%OM)
Corn grain	1.3	8.6	4.8	11.4	76.6	2.4	4.0
Alfalfa	9.4	16.6	39.3	49.2	34.1	9.6	2.1
Corn stover							
Untreated	7.9	7.1	48.3	77.3	16.8	10.4	1.0
STP	6.6	6.6	59.9	88.1	6.9	13.5	0.4
OLP	8.7	3.2	72.6	81.9	16.0	13.4	0.7
OLP + STP	10.3	4.1	77.2	87.6	10.4	9.1	0.6
STP + OLP	8.3	3.1	75.4	87.1	10.4	7.2	0.8
Extractives	31.4	28.0	1.0	1.7	69.7	0.3	1.2

Overall, NDF increased whereas NFC and CP both decreased through pretreatment. Based on composition alone, OLP and STP negatively affected corn stover feed value; however, digestibility analysis provides a significantly different conclusion.

6.4.2 NDFD48

NDFD48 was measured using *in vitro* anaerobic fermentation. Figure 6-2 shows data for corn grain, alfalfa, and untreated and pretreated corn stover. Previous literature has reported that improving forage NDFD increases dry matter intake and milk yield in dairy cows (Oba & Allen, 1999). The corn grain and alfalfa standards had NDFD values (g NDF digested/100 g NDF fed) of 63.2 and 47.9, respectively. Raw corn stover NDFD (49.3) was similar to alfalfa. OLP alone improved NDFD to 79.0, whereas STP alone reduced NDFD to 43.9. STP + OLP corn stover (76.0) was slightly less digestible than OLP alone; however, OLP + STP corn stover was the most digestible (79.3).

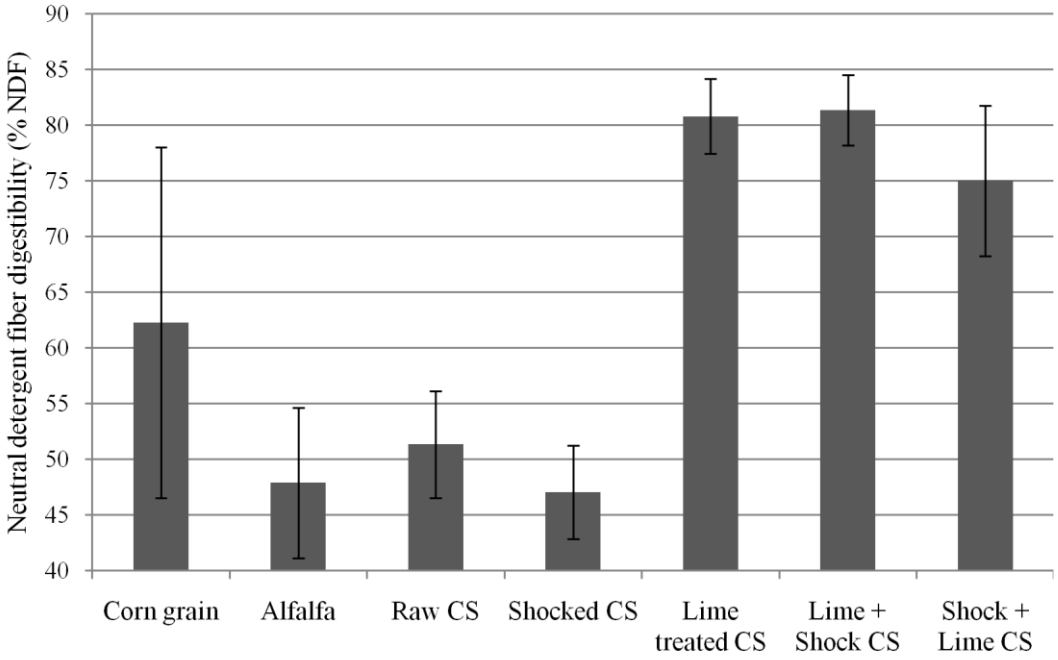

Figure 6-2. 48-h neutral detergent fiber digestibility of corn grain, alfalfa, and corn stover samples. (Errors bars are ± 1 standard deviations.)

6.4.3 TDN

The TDN of corn stover samples, corn grain standard, and alfalfa standard were estimated using two methods: (1) Weiss formula (Equation 6-1) using chemical analysis only (TDN_W), and (2) modified Weiss formula (Equation 6-4), which incorporates experimentally measured NDFD48 (TDN_N). Table 6-3 shows the calculated TDN values using each method on both a DM and OM basis. In this section, all TDN results discussed are presented as g nutrients digested/100 g OM fed.

Table 6-3. Total digestible nutrients of corn grain, alfalfa, and corn stover samples.

Sample	TDN_W (%DM)	TDN_W (%OM)	TDN_N (%DM)	TDN_N (%OM)
Corn grain	86.0	87.1	87.0	88.1
Alfalfa	55.7	61.5	53.9	59.4
Corn stover				
Untreated	48.1	52.2	47.8	51.9
STP	40.9	43.8	37.5	40.2
OLP	42.7	46.7	54.5	59.7
OLP + STP	45.2	50.3	62.5	69.7
STP + OLP	49.7	54.2	66.6	72.6
Extractives	61.0	88.9	NR	NR

NR = not reported

Because of its high NFC content, corn grain had the highest TDN_W (87.1) and TDN_N (88.1). Both methods estimated comparable values for alfalfa (61.5 and 59.4) and corn stover (52.2 and 51.9) for TDN_W and TDN_N, respectively. Because of the low NDFD for STP corn stover, the models resulted in similar values: 43.8 (TDN_W) and 40.2 (TDN_N).

As discussed previously, OLP, OLP + STP, and STP + OLP increased NDFD, resulting in significant differences between the two TDN estimation methods. In all three cases, TDN_N was much greater than TDN_W, because it accounts for the improved NDFD resulting from pretreatment. TDN_N was 59.7 for OLP corn stover, and 69.7 for OLP + STP corn stover. Of the corn stover samples, STP + OLP had the highest TDN_N (72.6), a difference of 18.4 from the TDN_W value. These modified TDN values show the effectiveness of the pretreatment processes, and demonstrate that traditional forage empirical models cannot predict the feed value of highly digestible lignocellulose.

6.4.4 In vitro gas production

During 48-h *in vitro* anaerobic fermentation used to measure NDFD, a pressure sensor was attached to the incubation flask to measure gas production. Gas production, displayed in Figure 6-3, can be correlated to fermentation rate. Figure 6-4 shows the rate of nutrient digestion, calculated by combining TDN_N and gas production. From gas production data, the fractional rate of fermentation was calculated using the following equation (Tedeschi et al., 2009):

$$V = V_F\{1 - e^{[-kf(t-\lambda)]}\} \quad (6\text{-}8)$$

where:

V = cumulative gas volume (mL)

V_F = gas volume corresponding to complete matter digestion (asymptote)

kf = fractional rate of fermentation (h^{-1})

t = time (h)

λ = lag time (h)

Table 6-4 displays the fractional rate of fermentation for each sample. As expected because of its high NFC content, corn grain had the highest fractional rate of fermentation (0.17/h). Raw corn stover had a low fractional rate (0.04/h), whereas STP + OLP improved the fractional rate (0.13/h).

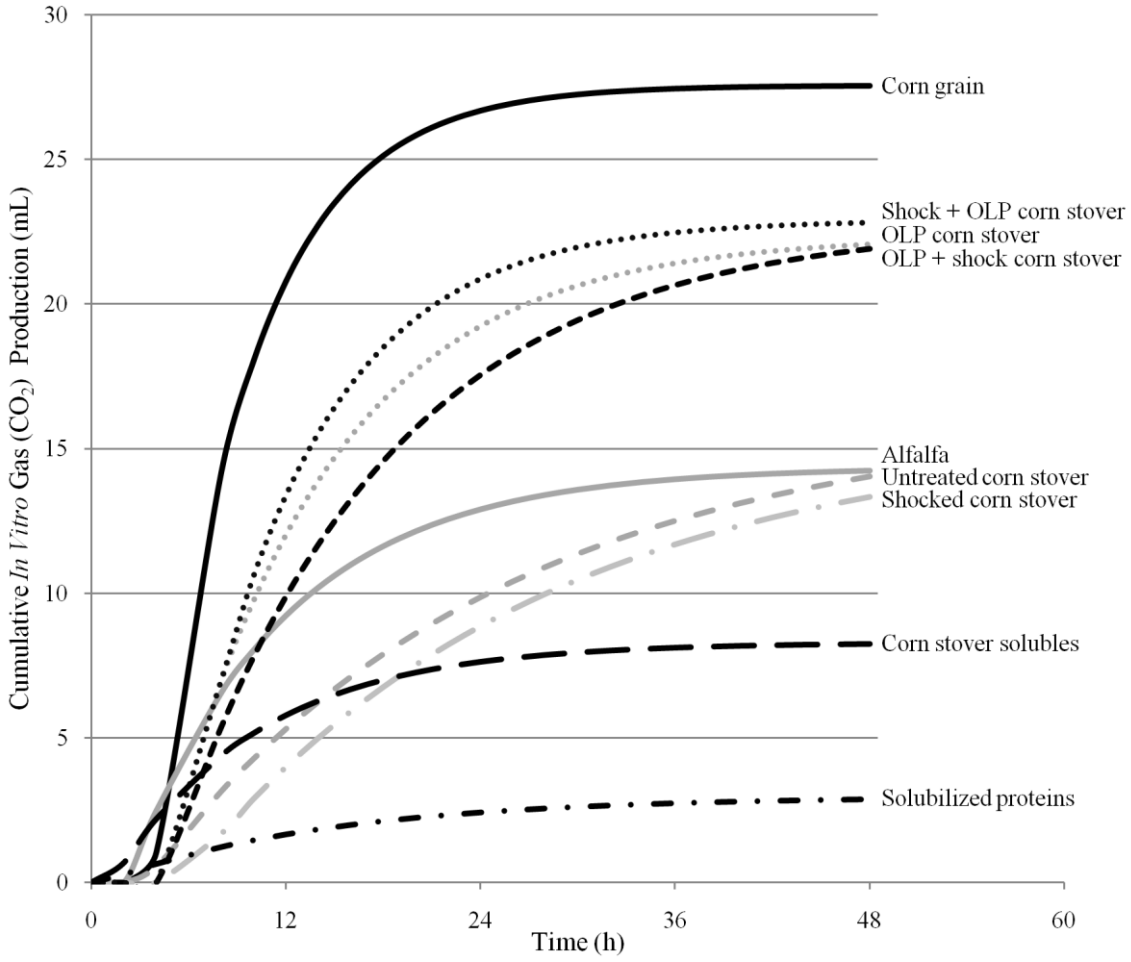

Figure 6-3. Gas production (mL) of corn grain, alfalfa, and corn stover samples during *in vitro* anaerobic fermentation.

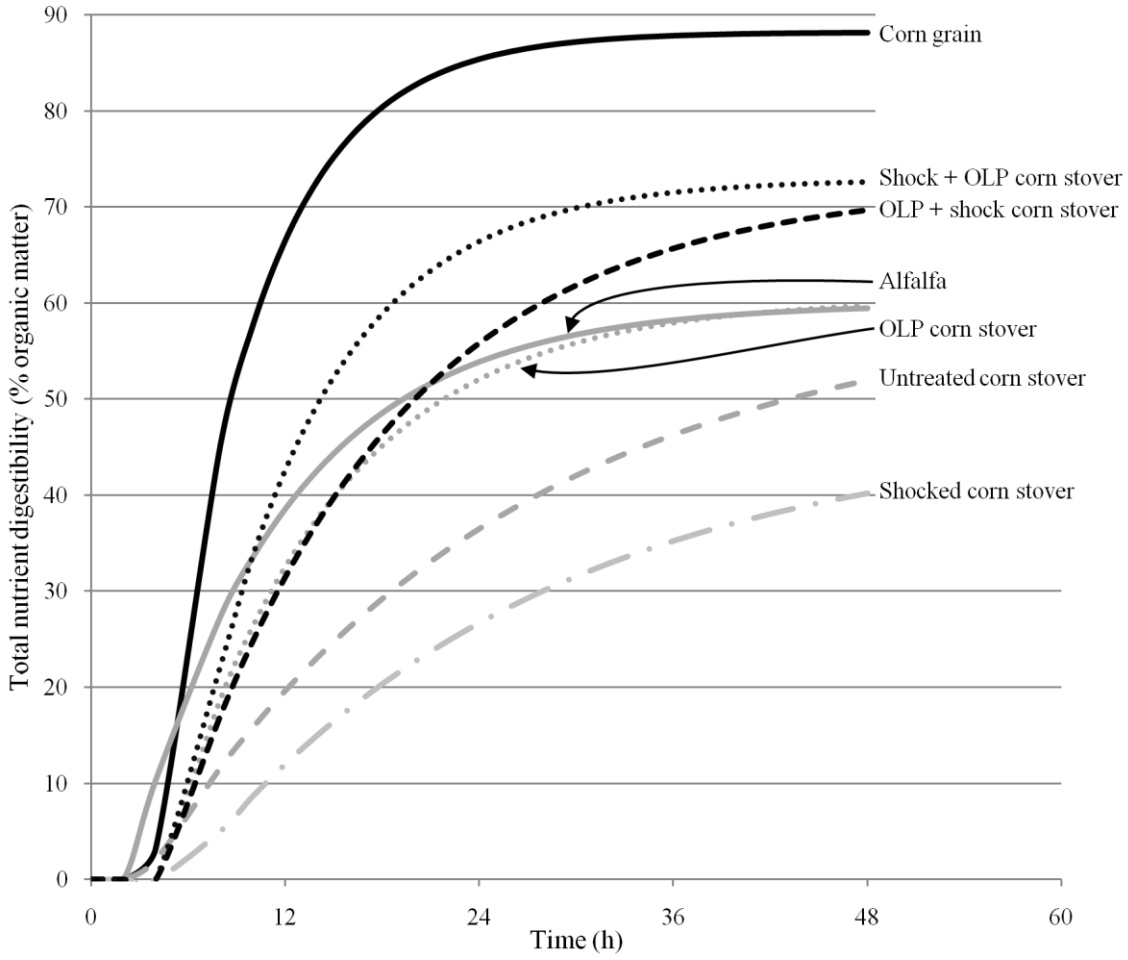

Figure 6-4. Total nutrient digestion rate of corn grain, alfalfa, and corn stover samples calculated using *in vitro* gas production and TDN_N on an OM basis.

Table 6-4. Fractional rate of fermentation (*kf*).

Sample	Fractional rate of fermentation (h^{-1})
Corn grain	0.17
Alfalfa	0.11
Corn stover	
Untreated	0.05
STP	0.05
OLP	0.10
OLP + STP	0.08
STP + OLP	0.13
Extractives	0.11

6.4.5 Addition of soluble extractives

As described in Section 6.2.1, raw corn stover was thoroughly washed with hot water to extract soluble components (approximately 14% by dry weight). Table 6-2 shows the composition of the extractives. The extractives had a TDN_W (g nutrients digested/100 g nutrients fed) of 61.0 on a dry matter basis, or 88.9 on an OM basis. [Note: NDFD48 was not determined for the extractives, so TDN_N could not be calculated; however, the NDF content was so low the two TDN methods should produce comparable values.]

Figure 6-5 shows a mass balance for each process step on a DM basis. Untreated corn stover contained 14% soluble extractives. OLP solids yield was 75%, thus 65% of untreated corn stover became OLP corn stover. As a mechanical pretreatment, STP does not affect the mass balance. Combining the corn stover sample with the highest TDN_N (STP + OLP) with extractives is 17.8% extractives and 82.2% STP + OLP.

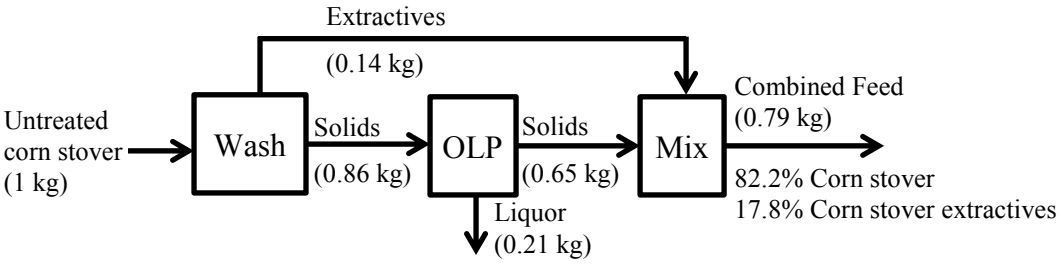

Figure 6-5. Mass balance for combining oxidative lime and shock tube-pretreated corn stover with pre-washed corn stover soluble extractives.

On a dry matter basis, STP + OLP corn stover had a TDN_N of 66.6, and the extractives had a TDN_W of 61.0. Their combined TDN was calculated as follows:

$$\text{TDN of combined feed} = (0.822)(66.6) + (0.178)(61.0) = 65.6$$

This combined TDN (65.6 g nutrients digested/100 g nutrients fed) shows a slightly negative effect from adding extractives to the treated corn stover, and is considerably lower than corn grain (–20.5). This results from the high ash content of the extractives. On an ash-free basis, the combined TDN was calculated as follows:

$$\text{TDN} = \frac{(0.822)(66.6) + (0.178)(61.0)}{(0.822)(1–0.083) + (0.178)(1–0.314)} = 74.9$$

This compares more favorably to ash-free corn grain (–12.3).

6.5 Conclusion

Combining OLP with STP improved the 48-h neutral detergent fiber digestibility to 79.0 g NDF digested/100 g NDF fed, compared to 49.3 for raw corn stover. STP did not further improve NDFD, but did increase TDN. On an OM basis, STP + OLP corn stover had a TDN of 72.6. When extractives were added, TDN increased to 74.9, which was only 13.2 less than corn grain.

CHAPTER VII

CONCLUSIONS AND RECOMMENDATIONS

In Chapter II, fermentation of shredded office paper was performed at a concentration of 100 g/L. Over the duration of fermentation, selectivity was not constant. To provide data useful for CPDM revision, similar fermentations should be performed at 20, 40, 70, and 100 g/L concentrations (with 20 g/L carboxylate salts added to 100 g/L fermentations, see Section 2.1).

Addition of 0.01 g calcium or sodium hydroxide/g fermentation sample effectively prevented product acid vaporization during drying at 105°C for 24 h. The change in measured MC between 0 and 0.01 g base/g fermentation sample was about 1.5%. As stated in Section 3.1, a large error on solid mass is produced by a small MC measurement error. Although the primary concern was to prevent product acid vaporization, no attempts were made to analyze vaporization of other components during drying.

After this and other research, shock tube mechanism and key variables remain largely unknown. Although results suggested that a 2-L fill volume and 00 shotgun shell should be used to increase enzymatic digestibility, more experimentation is needed. This study suggested that varying solids concentration between 0.02 and 0.10 g bagasse/g H_2O had no significant impact on performance. To revise process economics, determination of solids concentration effects on enzymatic digestibility and sugar yield are necessary.

After 42 days, fixed-bed batch fermentation of OLP + STP sugarcane bagasse produced acid concentrations of about 7 g/L and exit yields of about 0.1 g acid/g $NAVS_{in}$. Poor performance was due to short solid-liquid contact time, poor pH control, and the absence of mixing. Solid-liquid contact could be increased by decreasing percolation time into the liquid collection pipe. Improved pH control could be achieved using a dual buffer system in place of basic compounds. Poor mixing was a result of the type of fermentor system used, and energy savings from not mixing should be analyzed against discount in fermentation performance compared to well-mixed system.

Untreated and pretreated corn stover were compared to alfalfa and corn grain on the basis of composition, *in vitro* digestibility, and TDN. Although the compositions of pretreated corn stover samples (high NDF, low NFC) indicate low digestibility, STP + OLP bagasse and OLP + STP bagasse were more digestible than alfalfa. When water-soluble corn stover extractives were added, corn stover was only 13.2 g total digestible nutrients/100 g biomass less digestible than corn grain on an OM-basis.

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APPENDIX A

INOCULUM COLLECTION

1. Locate an area on the shoreline approximately 10 m from the tide.

2. Using a shovel, dig a 0.5 m-deep hole.

3. Collect liquid at the bottom of the hole into pre-labeled 1-L Beckman bottle(s).

4. Store bottle(s) in freezer.

APPENDIX B

DE-OXYGENATED WATER PREPARATION

1. Add 4-L DI H_2O to an Erlenmeyer flask.
2. Add 1.55 g sodium cysteine and 1.55 g sodium chloride to the flask.
3. Place aluminum foil over top of flask. Cut small holes in the foil.
4. Place flask on a hot plate and turn heating to HI.
5. After boiling for 1 h, turn off hot plate.
6. Allow DO H_2O to cool before storing.

APPENDIX C

CARBOXYLIC ACID QUANTIFICATION

1. If samples are frozen, allow them to thaw.

2. Vortex each sample for 5–10 s.

3. Place samples in centrifuge bucket adaptor (12 per adaptor).

4. Centrifuge samples at 4,000 rpm (3,297 × *g*) and 10°C for 25 min.

5. Pipette 0.5 mL each of sample, 3-M phosphoric acid, and 1.162 g/L iso-valeric acid into a 2-mL Eppendorf tube.

6. Centrifuge Eppendorf tubes at 15,000 rpm for 2 min.

7. Transfer 0.5 mL liquid from Eppendorf tube to labeled autosampler vial (National Scientific, No. C4011–5).

8. Secure vial caps to vials.

9. Prepare one vial containing external standard (ESTD) solution (10 μM acetic acid, *n*-propionic acid, *i*-propionic acid, *n*-butyric acid, *i*-butyric acid, *n*-valeric acid, *i*-valeric acid, *n*-caproic acid, and *n*-enanthic acid; Matreya LLC, No. 1075).

10. Analyze samples using a GC equipped with a flame ionization detector and a 30-m fused-silica capillary column (J&W Scientific Model No. 123-3232). Use an initial oven temperature of 40°C, ramp rate of 20°C/min to 200°C, and hold temperature at 200°C for 2 min.

APPENDIX D

145-mL OLP OPERATING PROCEDURE

1. Fill reactor(s) with 8 g biomass, 4 g calcium hydroxide, and 120 mL DI H_2O.

2. Using a pipe wrench, tightly close reactor cap(s). Place Teflon tape around pipe threads.

3. Place reactor(s) in stand inside oven and connect to O_2 line.

4. Set convection oven to the desired temperature, and power on swinging arm assembly.

5. Once oven temperature reaches desired temperature, open O_2 cylinder and adjust pressure regulator to desired pressure. This is the start of the reaction time.

6. At the end of the reaction, power off the oven, power off the swinging arm assembly, and close the O_2 regulator and cylinder. Relieve O_2 line by opening bleed valve.

7. Using about 500 mL DI H_2O, cool the reactor(s) and O_2 supply lines.

8. Carefully disconnect reactors from O_2 line and cool in an ice bath.

9. Once cool, unscrew reactor cap(s) and transfer reactor contents to a vacuum filtration apparatus. Using about 250 mL DI H_2O, transfer all reactor contents.

10. Follow Appendix G for neutralization and washing of OLP biomass.

APPENDIX E

8-L OLP OPERATING PROCEDURE

1. Fill reactor with 310 g biomass, 155 g calcium hydroxide, and 4,650 mL DI H_2O.

2. To wet biomass, mix reactor contents with a flat spatula.

3. Using two half-round plates and ten bolts, close and secure reactor.

4. Connect reactor to O_2 line, Magdrive supply and return water lines, and internal coil supply and return cooling water lines. Place thermocouple in thermowell.

5. Turn on reactor controller display.

6. Supply at least 0.5 L/min water through Magdrive supply line.

7. Turn on stirring, set to 30–50% maximum velocity.

8. Set temperature controller to desired set point.

9. Turn on heating power.

10. Once reactor reaches set point temperature, open O_2 cylinder and set pressure regulator to 6.9 bar. This is the initiation of the reaction time.

11. When the reaction time has elapsed, close the O_2 cylinder, turn off heating power, and begin cooling water flow through the reactor coil.

12. Once reactor is depressurized, open ball valve.

13. Turn off Magdrive supply water and turn off stirring.

14. Remove half-round plates and disconnect reactor from O_2 line, water, and cooling water lines.

15. Using DI H_2O, remove biomass from the internal coil.

16. Using a two-person crew, empty reactor contents into two or more gallon-sized buckets. To effectively remove all biomass, rinse reactor with DI H_2O.

17. Follow Appendix G for neutralization and washing of OLP biomass.

APPENDIX F

20-L OLP OPERATING PROCEDURE

1. Place 500 g biomass, 250 g calcium hydroxide, and 7,500 mL DI H_2O inside reactor.

2. Close reactor and tighten flange bolts.

3. Turn on stirring and heating mechanisms.

4. Once reactor has reached set point temperature, open O_2 cylinder and set regulator to 6.9 bar. This is the initiation of the reaction time.

5. When reaction time has elapsed, turn off heating and stirring.

6. Wait for reactor depressurization.

7. Open reactor.

8. Remove biomass from stirring mechanism and place in 5-gal container.

9. After placing 5-gal container below reactor, open reactor drain to collect biomass.

10. Until biomass has been recovered, rinse inner reactor surface with DI H_2O and collect under drain.

11. Follow Appendix G for neutralization and washing of OLP biomass.

APPENDIX G

NEUTRALIZATION AND WASHING OF OLP BIOMASS

1. Using vacuum filtration or a 140-mesh sieve, remove water and soluble components from biomass.

2. Place biomass in one or more 4-L plastic buckets. For each bucket, add a stir bar and place on a magnetic stir plate.

3. To each bucket, add 4-L DI H_2O.

4. Begin stirring biomass with magnetic stir bar.

5. Slowly add 5-N hydrochloric acid to each bucket until pH reaches 7. Allow 30 min for equilibration after acid addition. Record acid volume added. Monitor pH often.

7. Continue adding 5-N hydrochloric acid until pH reaches 4.

8. Using a 140-mesh sieve, remove water from biomass.

9. To each bucket, add 4-L DI H_2O.

10. Stir each bucket for a minimum of 5 min.

11. Repeat Steps 8–10 twice.

12. Using a 140-mesh sieve, remove water from biomass.

13. Place biomass in a labeled container. Container should be large enough to hold biomass at a depth no larger than 2 cm.

14. Stir biomass at least once every 24 h to ensure proper drying.

Calculations

1. Calcium hydroxide consumption is calculated by:

$$W_{Ca(OH)_2} = \frac{1\,\text{mol}\,Ca(OH)_2 \times N_{HCl} \times V_{HCl} \times M_{Ca(OH)_2}}{2\,\text{mol}\,HCl \times 1000\,\text{mL/L}}$$

where:

N_{HCl} = normality of acid (5 mol equivalents/L)

V_{HCl} = volume of acid added (mL)

$M_{Ca(OH)2}$ = molecular weight of calcium hydroxide (74.1 g/mol)

APPENDIX H

STP OPERATING PROCEDURE

1. For each STP, place desired mass of biomass into labeled plastic bag.

2. Take the following items to the shock tube site: goggles, gloves, paper towels, thermometer, ice, 2-L graduated cylinder, 140-mesh sieve, 5-gal bucket (or other container), large plastic tote. Items that have been provided by Gooseneck include: shotgun shells, flange gaskets, common hardware tools.

3. Upon arrival at the shock tube site, connect to water and air utilities.

4. Fill the plastic tote 2/3 full with water.

5. Connect air hose to impact wrench connected to 15/16-in socket.

6. Rinse inside of shock tube.

7. Place shock tube inside plastic tote.

8. Fill graduated cylinder to total desired volume.

9. Add a portion of the water to the biomass in the plastic bag.

10. Close the plastic bag and mix contents.

11. After the biomass has been soaked, carefully add it to the shock tube.

12. Using some of the remaining water, rinse out the plastic bag into the shock tube.

13. Add remaining water to the shock tube.

14. Lower top portion of shock tube.

15. Using steel bolts and nuts, tighten upper flange. Use of the impact wrench for this step is highly recommended.

16. Place shotgun shell with the brass facing upward at the top of the barrel.

17. Secure firing pin in place.

18. Compress spring using safety plate.

19. Fit triangular plate under safety plate and withdraw safety plate carefully.

20. Standing 20 feet from shock tube, pull string connected to triangular plate. This will fire the shotgun shell.

21. Remove firing pin.

22. With a razor blade and pliers, carefully remove shotgun shell. Shotgun shell may be under pressure.

23. Remove flange bolts.

24. Raise top portion of the shock tube.

25. Dump shock tube contents into large container.

26. Using 140-mesh sieve, remove water from biomass.

27. Place biomass in product container.

28. Repeat Steps 8–27 for remaining STP.

APPENDIX I

DETERMINATION OF MOISTURE CONTENT

This procedure is adapted from NREL procedure "Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples" (Sluiter, et al., 2008a).

1. Place weighing containers in oven set to 105°C for 24 h.
2. Remove containers from oven and immediately place them in a desiccator.
3. After containers cool to room temperature, weigh and record container masses.
4. Repeat Steps 1–3 until container mass difference is less than 0.001 g.
5. Place 0.5–2.0 g sample(s) in each container.
6. Place containers in oven set to 105°C for 24 h.
7. Remove containers from oven and immediately place them in a desiccator.
8. After containers cool to room temperature, weigh and record container masses.
9. Repeat Steps 6–8 until mass difference is less than 0.001 g.

APPENDIX J

DETERMINATION OF ASH CONTENT

This procedure is adapted from NREL procedure "Determination of Ash in Biomass" (Sluiter, et al., 2005a).

1. Place weighing containers in a muffle furnace set to 550°C for 4 h.
2. Remove containers from furnace and immediately place them in a desiccator.
3. After containers cool to room temperature, weigh and record container masses.
4. Repeat Steps 1–3 until container mass difference is less than 0.001 g.
5. Place 0.5–2.0 g sample(s) in each container.
6. Place containers in furnace set to 550°C for 4 h.
7. Remove containers from furnace and immediately place them in a desiccator.
8. After containers cool to room temperature, weigh and record container masses.
9. Repeat Steps 6–8 until mass difference is less than 0.001 g.

APPENDIX K

DETERMINATION OF EXTRACTIVES IN BIOMASS

This procedure is adapted from NREL procedure "Determination of Extractives in Biomass" (Sluiter, et al., 2005b).

1. Place 500 mL evaporation flask(s) in an oven set to 105°C for 24 h.

2. Remove flask(s) from oven and place immediately in a desiccator.

3. After cooling to room temperature, weigh and record mass of each flask as w_1.

4. Repeat Steps 1–3 until flask mass difference is less than 0.001 g.

5. Add 5–10 boiling stones and 300 mL solvent to each flask. Ethanol and DI H_2O are most commonly used.

6. Weigh 4–8 g biomass onto a large coarse filter paper. Record mass as w_3.

7. To contain biomass during extraction, wrap filter paper around biomass.

8. Place each filter paper in a cellulose thimble (Whatman, double thickness) and place in a Soxhlet tube.

9. Set up Soxhlet extraction. Place items (bottom to top): heating mantle, evaporation flask, Soxhlet tube, condenser. Connect condensers to circulating cooling water.

10. Turn on heating mantles and circulating cooling water.

11. Maintain extraction for 16–24 h. Reflux events, in which condensed solvent in Soxhlet tube returns to evaporation flask, should occur each 8–12 min.

12. Turn off heating mantles and circulating cooling water.

13. Once glassware has cooled to room temperature, collect cellulose thimbles and evaporation flasks.

14. Before use, allow biomass to dry.

15. Using a Rotavap set to 40°C connected to a vacuum pump, slowly boil solvent from evaporation flasks.

16. Once solvent is removed, place flasks in an oven set to 40°C for 24 h.

17. Remove flasks from oven and place immediately in a desiccator.

18. Weigh and record flask mass as w_2.

19. Repeat Steps 17–19 until mass difference is less than 0.001 g.

Calculations

1. Calculate mass fraction extractives by:

$$\text{Extractives} = \frac{w_2 - w_1}{(w_3)(1 - \text{MC})}$$

where:

w_1 = flask weight (g)

w_2 = flask + extractives weight (g)

w_3 = biomass weight (g)

MC = sample moisture content

APPENDIX L

DETERMINATION OF STRUCTURAL CARBOHYDRATES AND LIGNIN IN BIOMASS USING ACID HYDROLYSIS

This procedure is adapted from NREL procedure "Determination of Structural Carbohydrates and Lignin in Biomass" (Sluiter, et al., 2008b).

1. Using Appendix I, determine moisture content of biomass samples. Moisture content should be less than or equal to 0.10.

2. For untreated samples, perform Appendix K to remove extractives prior to this analysis.

3. Prior to analysis, biomass should be ground to –20/+80 mesh size.

4. Place filtering crucibles (Coors, No. 60531) in muffle furnace set to 550°C for 4 h.

5. Remove filtering crucibles from the furnace and place immediately in a desiccator.

6. After cooling to room temperature, weigh crucible mass.

7. Repeat Steps 4–6 until mass difference is less than 0.001 g.

8. Performing triplicate analysis is strongly advised. For each sample, add 0.30 ± 0.01 g to a labeled test tube (16×100 mm or larger). Record sample weights as w_1.

9. To each test tube, add 3 mL 72% sulfuric acid.

10. Place test tubes in a water bath set to 30°C for 60 min, stirring every 5–10 min with a glass stir rod.

11. Remove test tubes from water bath.

12. Using 84 mL DI H_2O, quantitatively transfer test tube contents to labeled glass autoclave bottles. Tighten a crimp cap on each bottle.

13. In a labeled glass autoclave bottle, prepare sugar recovery standard (SRS). Add amounts of each sugar being analyzed representative of the amounts present in the samples. To the bottle, add 84 mL DI H_2O and 3 mL sulfuric acid. Tighten crimp cap on bottle and shake contents. Immediately open bottle and transfer 15 mL into a labeled 50-mL conical vial. Replace crimp cap and perform Steps 14–18 on SRS as with other samples.

14. Autoclave samples at 121°C for 1 h.

15. Allow bottles to slowly cool to room temperature.

16. Using 1-L Erlenmeyer flasks, filtering crucibles, and crucible adapters, vacuum filter bottle contents.

17. Transfer 15 mL filtrate to a labeled conical vial.

18. Add calcium carbonate to neutralize 15 mL liquid.

19. Prior to carbohydrate analysis by HPLC, store vials in freezer.

20. Using 50 mL hot DI H_2O, transfer biomass residue from glass bottle to filtering crucible.

21. Place filtering crucibles in an oven set to 105°C for 24 h.

22. Remove crucibles from oven and immediately place them in a desiccator.

23. After cooling to room temperature, weigh and record mass as w_2.

24. Repeat Steps 20–22 until mass difference is less than 0.001 g.

25. Place filtering crucibles in a muffle furnace set to 550°C for 4 h.

26. Remove crucibles from furnace and immediately place them in a desiccator.

27. After cooling to room temperature, weight and record mass as w_3.

28. Thaw neutralized samples.

29. Vortex each sample for 5–10 s.

30. Place vials in centrifuge adapters.

31. Centrifuge at 4,000 rpm (3,297 × *g*) for 5 min.

32. Using a 1-mL syringe and 0.2-µm syringe filter, transfer liquid from vial to a labeled glass HPLC vial. Cap the vial.

33. Prepare calibration standards for applicable sugars. Common sugar calibrations are 0.5, 1, 2, 5, and 10 g/L.

34. Analyze calibration standards, two SRS samples, and biomass samples. Use an Aminex HPX-87P column, guard column, and refractive index detector. Use a degassed, 0.2-µm-filtered DI H_2O mobile phase, 20 µL injection volume, and 20 min run time.

Calculations

1. Acid insoluble lignin (AIL) is calculated by:

$$\text{AIL} = \frac{w_2 - w_3}{w_1(1 - \text{MC})}$$

where:

w_1 = sample weight (g)

w_2 = filtering crucible plus dry residue (g)

w_3 = filtering crucible plus ash (g)

MC = sample moisture content

2. Mass fractions for each sugar (x_i) are calculated by:

$$x_i = \frac{\text{R}_{\text{SRS}_i} \times C_i \times \text{AHC}_i \times 87\ \text{mL}}{w_1 \times (1 - \text{MC}) \times 1000\ \text{mL/L}} \tag{4-3}$$

where:

C_i = HPLC-determined sugar concentration for Sugar i (g/L)

AHC_i = anhydro correction for component i (0.90 for glucan, 0.88 for xylan)

R_{SRSi} = recovery of sugar recovery standard for Sugar i

w_1 = sample weight (g)

3. Sugar recovery standard recovery for each sugar (R_{SRSi}) calculated by:

$$R_{SRS_i} = \frac{SRS_{2_i}}{SRS_{1_i}}$$

where:

SRS_{2i} = final SRS concentration for Sugar *i* (g/L)

SRS_{1i} = initial SRS concentration for Sugar *i* (g/L)

APPENDIX M

DETERMINATION OF BIOMASS DIGESTIBILITY USING ENZYMATIC HYDROLYSIS

This procedure is adapted from NREL procedure "Enzymatic Saccharification of Lignocellulosic Biomass" (Selig, et al., 2008).

1. Using Appendix I, determine moisture content of biomass samples. Moisture content should be less than or equal to 0.10.

2. For untreated samples, perform Appendix K to remove extractives prior to this analysis.

3. Prior to analysis, biomass should be ground to –20/+80 mesh size.

4. To each conical vial, add the amount of biomass (B) equivalent to 0.1 g glucan:

$$B = \frac{0.1}{G(1 - MC)}$$

where:

G = glucan mass fraction

MC = moisture content

5. Add 5 mL 0.1-M citric acid solution, 40 µL tetracycline solution (10 g/L in 70% ethanol), and 30 µL cycloheximide solution (10 g/L in DI H_2O).

6. Calculate addition of cellulase E_1 and cellobiase E_2:

$$E_1 = \frac{0.1L_1}{Y_G E_{A_1}}$$

where:

L_1 = cellulase loading (FPU/g glucan in raw biomass; typical values are 5 and 15)

Y_G = pretreatment yield of glucan

E_{A1} = enzyme activity (FPU/mL)

$$E_2 = \frac{0.1L_2}{Y_G E_{A_2}}$$

where:

L_2 = cellobiase loading (CBU/g glucan in raw biomass; typical)

E_{A2} = cellobiase activity (CBU/mL)

7. Add DI H_2O to conical vial(s). Biomass and other chemicals are assumed to have a density of 1 g/mL. Calculate water addition (W) by:

$$W = 10 - 5 - 0.04 - 0.03 - B - E_1 - E_2$$

8. Place conical vial(s) at a 45° angle in shaking incubator set to 50°C and 105 rpm for 1 h.

9. Add cellulase and cellobiase enzymes and place vial(s) back in incubator for desired time period (6, 24, and 72 h are typical).

10. To denature enzymes, remove vial(s) and place them in an oven set to 105°C for 10 min.

11. Store vial(s) in the freezer.

12. Prepare samples for HPLC analysis as described in Steps 33 and 34 of Appendix L.

Calculations

1. Enzymatic digestibility is calculated by:

$$\text{Digestibility} = \frac{(V_{EH})(C_{glu,eh} \times \text{AHC}_{\text{glu}} + C_{xyl,eh} \times \text{AHC}_{\text{xyl}})}{\text{B}(1 - \text{MC})(x_{glu,p} + x_{xyl,p})(1000\ \text{mL/L})} \quad (4\text{-}4)$$

where:

V_{EH} = enzymatic hydrolysis total volume (10 mL)

$C_{glu,eh}$ = HPLC-determined glucose concentration after enzymatic hydrolysis (g/L)

$C_{xyl,eh}$ = HPLC-determined xylose concentration after enzymatic hydrolysis (g/L)

B = mass of bagasse loaded (g)

$x_{glu,p}$ = mass fraction glucan in the pretreated sample

$x_{xyl,p}$ = mass fraction xylan in the pretreated sample

2. Sugar yield is calculated by:

$$\text{Yield} = \frac{(\text{V}_{\text{EH}})(C_{glu,eh} \times \text{AHC}_{\text{glu}} + C_{xyl,eh} \times \text{AHC}_{\text{xyl}})}{\text{B}(1 - \text{MC})(x_{glu,p}/Y_G + x_{xyl,p}/Y_P)(1000\ \text{mL/L})} \quad (4\text{-}5)$$

where:

Y_G = pretreatment glucan yield (g glucan in pretreated/g glucan in raw)

Y_P = pretreatment xylan yield (g xylan in pretreated/g xylan in raw)

APPENDIX N

INVESTIGATION OF THE CPDM CONSTANT-SELECTIVITY ASSUMPTION

DATA

Table N-1. Fermentor mass balances.

	Mass In (g)					**Mass Out** (g)						
Fermentor	Acid	Water	NAVS	Ash	Total	Gas	Acid	Water	Water of Hydrolysis	NAVS	Ash	Total
1-1	0.2	402	30.3	14.0	446.5	0.5	0.3	404.4	0.4	27.0	13.2	445.8
1-2	0.2	402	30.3	14.0	446.5	0.4	0.3	408.5	0.8	23.5	11.3	444.8
1-3	0.2	402	30.4	14.0	446.5	0.4	0.3	406.9	0.6	25.2	12.8	446.1
2-1	0.2	402	30.4	14.0	446.5	0.9	0.4	407.8	0.6	25.0	12.0	446.7
2-2	0.1	402	30.4	14.0	446.5	3.4	1.0	404.2	0.8	23.3	12.7	445.4
2-3	0.2	402	30.3	14.0	446.5	1.2	0.6	408.2	0.7	24.2	12.6	447.6
3-1	0.1	402	30.4	14.0	446.5	3.3	1.7	401.0	0.7	24.0	13.7	444.6
3-2	0.2	402	30.3	14.0	446.5	1.9	0.6	400.8	0.0	28.2	14.5	446.0
3-3	0.2	402	30.4	14.0	446.5	4.2	2.4	400.7	1.1	20.8	13.4	442.5
4-1	0.1	402	30.4	14.0	446.5	5.2	3.6	403.7	1.3	18.6	13.5	446.0
4-2	0.1	402	30.4	14.0	446.5	5.4	3.9	401.0	1.3	18.4	14.2	444.2
4-3	0.1	402	30.4	14.0	446.5	5.8	4.3	402.9	1.5	16.9	15.4	446.7
5-1	0.2	402	30.3	14.0	446.5	7.9	4.5	400.1	1.4	17.9	14.5	446.2
5-2	0.2	402	30.3	14.0	446.5	5.7	4.2	402.4	1.5	16.9	15.1	445.8
5-3	0.1	402	30.4	14.0	446.5	5.3	4.8	397.6	1.5	17.1	14.2	440.6
6-1	0.1	402	30.4	14.0	446.5	5.3	4.5	400.7	1.4	17.8	14.0	443.8
6-2	0.1	402	30.4	14.0	446.5	5.5	4.8	402.5	1.5	17.1	13.8	445.2
6-3	0.1	402	30.4	14.0	446.5	4.5	4.1	397.5	1.3	18.5	14.5	440.3
7-1	0.1	402	30.4	14.0	446.5	7.1	6.1	403.8	1.8	14.3	14.5	447.7
7-2	0.1	402	30.4	14.0	446.5	6.2	5.5	399.2	1.7	15.2	14.2	442.0
7-3	0.2	402	30.3	14.0	446.5	6.2	5.8	392.5	1.8	14.0	13.8	434.1
8-1	0.1	402	30.4	14.0	446.5	5.9	5.3	399.0	1.6	16.2	12.2	440.3
8-2	0.1	402	30.4	14.0	446.5	5.2	4.6	392.7	1.2	19.9	14.8	438.4
8-3	0.1	402	30.4	14.0	446.5	6.4	6.9	399.3	2.0	12.8	13.3	440.6
8-4	0.1	402	30.4	14.0	446.5	5.0	5.6	393.9	1.6	16.0	14.7	436.8

Table N-2. End of fermentation material accounting. (For each fermentor, first row MC, AC, and VSC pertain to liquid, whereas final three rows pertain to solid.)

Fermentor	Liquid Wt (g)	Cake Wt (g)	MC	AC (wet)	AC (dry)	VSC (dry)
1-1	177.9	258	0.992	0.003	0.394	0.606
1-1	---	---	0.853	0.047	0.319	0.681
1-1	---	---	0.849	0.051	0.335	0.665
1-1	---	---	0.843	0.049	0.315	0.685
1-2	170.3	264.3	0.994	0.003	0.425	0.575
1-2	---	---	0.868	0.043	0.324	0.676
1-2	---	---	0.880	0.039	0.320	0.680
1-2	---	---	0.867	0.042	0.314	0.686
1-3	169.8	266.3	0.994	0.002	0.421	0.579
1-3	---	---	0.862	0.045	0.326	0.674
1-3	---	---	0.867	0.044	0.327	0.673
1-3	---	---	0.852	0.051	0.343	0.657
2-1	176.3	253.9	0.993	0.003	0.488	0.512
2-1	---	---	0.865	0.044	0.326	0.674
2-1	---	---	0.862	0.043	0.308	0.692
2-1	---	---	0.845	0.049	0.315	0.685
2-2	210.2	216.0	0.986	0.006	0.398	0.602
2-2	---	---	0.850	0.050	0.331	0.669
2-2	---	---	0.839	0.057	0.351	0.649
2-2	---	---	0.839	0.054	0.335	0.665
2-3	174.3	256.4	0.992	0.004	0.451	0.549
2-3	---	---	0.867	0.044	0.331	0.669
2-3	---	---	0.871	0.045	0.348	0.652
2-3	---	---	0.840	0.052	0.322	0.678
3-1	223.9	195.6	0.984	0.006	0.408	0.592
3-1	---	---	0.827	0.061	0.354	0.646
3-1	---	---	0.811	0.062	0.329	0.671
3-1	---	---	0.812	0.065	0.345	0.655
3-2	138.4	284.6	0.989	0.005	0.441	0.559
3-2	---	---	0.847	0.049	0.323	0.677
3-2	---	---	0.848	0.053	0.346	0.654
3-2	---	---	0.865	0.043	0.322	0.678
3-3	244.3	172	0.980	0.008	0.382	0.618
3-3	---	---	0.819	0.067	0.371	0.629
3-3	---	---	0.812	0.067	0.359	0.641
3-3	---	---	0.816	0.066	0.361	0.639
4-1	291.2	121.2	0.975	0.013	0.507	0.493
4-1	---	---	0.784	0.067	0.311	0.689
4-1	---	---	0.762	0.081	0.340	0.660
4-1	---	---	0.750	0.096	0.382	0.618
4-2	273.9	136.5	0.974	0.012	0.478	0.522
4-2	---	---	0.803	0.066	0.335	0.665
4-2	---	---	0.778	0.080	0.358	0.642
4-2	---	---	0.774	0.093	0.410	0.590

Table N-2. Continued.

Fermentor	Liquid Wt (g)	Cake Wt (g)	MC	AC (wet)	AC (dry)	VSC (dry)
4-3	325	87.4	0.972	0.014	0.491	0.509
4-3	---	---	0.702	0.102	0.341	0.659
4-3	---	---	0.695	0.121	0.397	0.603
4-3	---	---	0.655	0.155	0.449	0.551
5-1	302.2	101.6	0.972	0.014	0.504	0.496
5-1	---	---	0.751	0.075	0.300	0.700
5-1	---	---	0.715	0.106	0.371	0.629
5-1	---	---	0.695	0.123	0.405	0.595
5-2	304.4	101.1	0.975	0.012	0.483	0.517
5-2	---	---	0.744	0.090	0.352	0.648
5-2	---	---	0.699	0.130	0.431	0.569
5-2	---	---	0.712	0.118	0.408	0.592
5-3	315.3	85.3	0.972	0.013	0.455	0.545
5-3	---	---	0.723	0.083	0.299	0.701
5-3	---	---	0.681	0.117	0.366	0.634
5-3	---	---	0.644	0.158	0.444	0.556
6-1	307.5	90.5	0.972	0.013	0.480	0.520
6-1	---	---	0.730	0.079	0.292	0.708
6-1	---	---	0.676	0.130	0.400	0.600
6-1	---	---	0.677	0.119	0.369	0.631
6-2	310.3	88.8	0.972	0.013	0.478	0.522
6-2	---	---	0.725	0.084	0.306	0.694
6-2	---	---	0.708	0.096	0.330	0.670
6-2	---	---	0.658	0.147	0.430	0.570
6-3	270.8	124.7	0.975	0.011	0.458	0.542
6-3	---	---	0.761	0.091	0.382	0.618
6-3	---	---	0.769	0.083	0.358	0.642
6-3	---	---	0.743	0.099	0.387	0.613
7-1	318	75.7	0.967	0.016	0.481	0.519
7-1	---	---	0.696	0.095	0.313	0.687
7-1	---	---	0.670	0.141	0.428	0.572
7-1	---	---	0.666	0.139	0.416	0.584
7-2	312.3	76.7	0.969	0.014	0.464	0.536
7-2	---	---	0.702	0.098	0.328	0.672
7-2	---	---	0.676	0.119	0.367	0.633
7-2	---	---	0.639	0.164	0.453	0.547
7-3	312	69	0.966	0.015	0.452	0.548
7-3	---	---	0.686	0.112	0.356	0.644
7-3	---	---	0.686	0.111	0.354	0.646
7-3	---	---	0.633	0.169	0.461	0.539
SP-1	289.9	91.9	0.972	0.013	0.461	0.539
SP-1	---	---	0.725	0.088	0.319	0.681
SP-1	---	---	0.729	0.083	0.307	0.693
SP-1	---	---	0.705	0.108	0.366	0.634
SP-2	271.3	109.7	0.970	0.012	0.402	0.598

Table N-2. Continued.

Fermentor	Liquid Wt (g)	Cake Wt (g)	MC	AC (wet)	AC (dry)	VSC (dry)
SP-2	---	---	0.666	0.127	0.381	0.619
SP-2	---	---	0.739	0.097	0.373	0.627
SP-2	---	---	0.742	0.090	0.351	0.649
SP-3	308.4	72.8	0.964	0.017	0.460	0.540
SP-3	---	---	0.709	0.104	0.356	0.644
SP-3	---	---	0.704	0.109	0.370	0.630
SP-3	---	---	0.688	0.123	0.396	0.604
SP-4	300.9	78.3	0.962	0.019	0.501	0.499
SP-4	---	---	0.666	0.132	0.394	0.606
SP-4	---	---	0.693	0.106	0.346	0.654
SP-4	---	---	0.695	0.104	0.341	0.659

APPENDIX O

MOISTURE CONTENT DATA

Table O-1. Base addition data.

Base	Base Conc.	Final pH	MC	% Acid Retained	AVG Base Conc.	SD Base Conc.	AVG MC	SD MC	AVG Acid Retained	SD Acid Retained
$Ca(OH)_2$	0.010	10.77	0.960	102.943						
$Ca(OH)_2$	0.010	10.83	0.963	97.340	0.010	0.000	0.961	0.001	97.641	5.159
$Ca(OH)_2$	0.010	10.67	0.961	92.639						
$Ca(OH)_2$	0.005	9.22	0.970	68.632						
$Ca(OH)_2$	0.005	9.22	0.970	65.762	0.005	0.000	0.970	0.001	69.396	4.070
$Ca(OH)_2$	0.005	9.32	0.970	73.794						
NaOH	0.005	9.24	0.971	67.740						
NaOH	0.005	9.24	0.974	62.025	0.005	0.000	0.971	0.003	66.645	4.182
NaOH	0.005	9.36	0.967	70.171						
NaOH	0.010	11.00	0.966	91.038	0.010	0.000	0.965	0.001	90.117	1.303
NaOH	0.010	11.08	0.964	89.195						
NaOH	0.015	13.02	0.964	99.393	0.015	0.000	0.964	0.000	99.772	0.536
NaOH	0.015	12.97	0.964	100.151						
None	0.000	6.58	0.983	39.710						
None	0.000	6.37	0.982	39.615	0.000	0.000	0.983	0.000	38.897	1.327
None	0.000	6.35	0.983	37.366						
$Ca(OH)_2$	0.015	12.65	0.963	99.310						
$Ca(OH)_2$	0.015	12.62	0.965	84.029	0.015	0.000	0.965	0.002	90.156	8.078
$Ca(OH)_2$	0.016	12.63	0.967	87.129						
$Ca(OH)_2$	0.020	12.65	0.968	103.197						
$Ca(OH)_2$	0.020	12.64	0.965	102.510	0.020	0.000	0.966	0.001	96.606	10.826
$Ca(OH)_2$	0.020	12.65	0.966	84.111						
NaOH	0.020	13.02	0.964	94.309						
NaOH	0.020	12.93	0.964	84.751	0.020	0.000	0.965	0.002	86.838	6.677
NaOH	0.020	12.89	0.967	81.453						
None	0.000	6.41	0.983	36.434						
None	0.000	6.40	0.982	38.547	0.000	0.000	0.983	0.001	37.545	1.061
None	0.000	6.40	0.983	37.654						

APPENDIX P

OLP AND STP OF SUGARCANE BAGASSE DATA

Table P-1. Sugarcane bagasse pretreatment data.

Sample	MC	x_{Glu}	x_{Xyl}	Enz. Hyd. Wt.	Yield (6 h)	Yield (24 h)	Yield (72 h)
Shock 1	0.067	0.496	0.225	0.2129	0.000	0.000	0.640
Shock 1	0.066	0.493	0.220	0.2129	0.000	0.000	0.641
Shock 1	0.062	0.518	0.215	0.2129	0.000	0.000	0.663
Shock 2	0.076	0.477	0.196	0.2259	0.000	0.000	0.717
Shock 2	0.080	0.000	0.000	0.2259	0.000	0.000	0.666
Shock 2	0.072	0.481	0.201	0.2259	0.000	0.000	0.628
Shock 3	0.071	0.469	0.196	0.2267	0.000	0.000	0.675
Shock 3	0.069	0.481	0.204	0.2267	0.000	0.000	0.613
Shock 3	0.073	0.475	0.198	0.2267	0.000	0.000	0.636
Shock 4	0.070	0.495	0.208	0.2206	0.000	0.000	0.605
Shock 4	0.071	0.480	0.206	0.2206	0.000	0.000	0.624
Shock 4	0.069	0.488	0.201	0.2206	0.000	0.000	0.654
Shock 5	0.067	0.490	0.210	0.2222	0.000	0.000	0.728
Shock 5	0.068	0.478	0.201	0.2222	0.000	0.000	0.659
Shock 5	0.068	0.481	0.199	0.2222	0.000	0.000	0.619
Shock 6	0.077	0.439	0.123	0.2485	0.000	0.000	0.808
Shock 6	0.073	0.445	0.135	0.2485	0.000	0.000	0.809
Shock 6	0.075	0.422	0.118	0.2485	0.000	0.000	0.793
Shock 7	0.068	0.53	0.167	0.2052	0.000	0.000	0.652
Shock 7	0.073	0.525	0.168	0.2052	0.000	0.000	0.640
Shock 7	0.070	0.517	0.193	0.2052	0.000	0.000	0.702
Shock 8	0.075	0.458	0.194	0.2469	0.000	0.000	0.815
Shock 8	0.075	0.425	0.140	0.2469	0.000	0.000	0.786
Shock 8	0.074	0.43	0.142	0.2469	0.000	0.000	0.823
Shock 9	0.073	0.497	0.156	0.2177	0.000	0.000	0.724
Shock 9	0.081	0.493	0.147	0.2177	0.000	0.000	0.685
Shock 9	0.078	0.503	0.152	0.2177	0.000	0.000	0.661
Shock 10	0.071	0.437	0.106	0.2441	0.000	0.000	0.885
Shock 10	0.072	0.449	0.116	0.2441	0.000	0.000	0.920
Shock 10	0.071	0.438	0.116	0.2441	0.000	0.000	0.937
Shock 11	0.080	0.484	0.143	0.2243	0.000	0.757	0.838
Shock 11	0.077	0.483	0.148	0.2243	0.000	0.734	0.775
Shock 11	0.079	0.000	0.000	0.2243	0.000	0.667	0.765
Shock 12	0.091	0.487	0.148	0.2242	0.000	0.664	0.787
Shock 12	0.086	0.473	0.143	0.2242	0.000	0.645	0.783
Shock 12	0.088	0.508	0.163	0.2242	0.000	0.664	0.700
Shock 13	0.079	0.400	0.103	0.2642	0.000	0.768	0.993
Shock 13	0.071	0.418	0.110	0.2642	0.000	0.819	0.908

Table P-1. Continued.

Sample	MC	x_{Glu}	x_{Xyl}	Enz. Hyd. Wt.	Yield (6 h)	Yield (24 h)	Yield (72 h)
Shock 13	0.073	0.409	0.108	0.2642	0.000	0.788	0.897
Shock 14	0.079	0.507	0.155	0.2181	0.000	0.638	0.700
Shock 14	0.074	0.486	0.152	0.2181	0.000	0.612	0.763
Shock 14	0.079	0.498	0.161	0.2181	0.000	0.669	0.654
STP	0.091	0.393	0.158	0.2817	0.000	0.092	0.039
STP	0.091	0.389	0.151	0.2817	0.000	0.091	0.060
STP	0.094	0.39	0.151	0.2817	0.000	0.100	0.018
STP + OLP	0.076	0.467	0.159	0.2334	0.000	0.539	0.606
STP + OLP	0.075	0.458	0.160	0.2334	0.000	0.550	0.600
STP + OLP	0.073	0.463	0.166	0.2334	0.000	0.611	0.555
Shock 16	0.076	0.411	0.099	0.2645	0.000	0.832	0.932
Shock 16	0.072	0.409	0.102	0.2645	0.000	0.885	0.880
Shock 16	0.078	0.407	0.103	0.2645	0.000	0.912	1.041
Shock 17	0.080	0.416	0.092	0.2592	0.000	0.924	0.916
Shock 17	0.077	0.422	0.096	0.2592	0.000	0.919	0.945
Shock 17	0.075	0.416	0.094	0.2592	0.000	0.878	0.935
Ex Free	0.062	0.429	0.202	0.2477	0.000	0.153	0.150
Ex Free	0.065	0.425	0.189	0.2477	0.000	0.156	0.143
Ex Free	0.064	0.439	0.200	0.2477	0.000	0.147	0.137
OLP Bulk	0.067	0.504	0.177	0.2093	0.000	0.569	0.613
OLP Bulk	0.069	0.525	0.189	0.2093	0.000	0.556	0.465
OLP Bulk	0.070	0.510	0.180	0.2093	0.000	0.541	0.594

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