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DEPARTMENT OF CHEMICAL ENGINEERING

OPTIMIZATION OF PH FOR ACIDOGENIC DIGESTION OF WILLOW TO MIXED ORGANIC ACIDS

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ABSTRACT

The carboxylate platform is a promising route for biofuels production, capable of converting a variety of biomass materials to liquid fuel without either sterile conditions or costly enzymes. Earlier work has investigated acidogenic digestion on material such as corn stover. Willow wood is less well-studied biomass. An acidogenic digestion of pretreated willow was conducted at multiple pH treatments from pH 4.5 to 6.5. Compositional analysis was conducted on pretreated starting material as well final solid residue. The digestion products quantified were mixed organic acids from C1 to C6. Steam explosion pretreatment was employed, with temperature at 190ºC and residence time at 50 seconds. A non-specified, mixed bacterial culture was used. High organic acid concentrations were achieved of up to 29.75 ± 0.80 g/L. Lower pH treatments produced low acid concentrations; production of acid was maximized at pH 6. Six organic acids accounted for greater than 99% of the acids produced: hexanoic, butyric, propionic, acetic, formic, and lactic acid. A correlation between concentration of acetic acid and pH treatment was observed to exist with 95% confidence. The digestion optimized reactor pH for a lignocellulosic biomass that shows promise for use in biofuel production. Development of a system for in-situ removal of acid products is a candidate for future studies.

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Chapter 1

Literature Review

1.1 Petroleum demand and supply

Each day, 660 million barrels of oil are used worldwide, a volume equivalent to more than 6,000 Olympic sized swimming pools (U.S. Energy Information Administration, 2014). Petroleum is heavily relied upon in economies worldwide; it heats buildings, is used in the production of innumerable chemicals, and fuels transportation. The amount of the substance used demonstrates the extent to which humankind relies upon it, and the risk inherent in high consumption with an immanent end to economically recoverable oil.

Oil is a resource that is being used greatly in excess of its ability to be naturally replenished. Oil is replenished on geologic timescales; humanity uses oil at rates that are exponentially higher. Over the last 100 years many of the easily recovered reservoirs of oil have been exploited, and new unconventional oil is found in deep offshore sites, polar regions, and tightly bound shales, all more expensive to develop. As a consequence of this, there is a finite amount of oil that can be recoverable economically. The cost of extraction will, at some point, exceed the potential value gained by its use. By current estimations, there are 50 years remaining of economically extractable oil (Bentley, 2002). As the amount of oil that is economically extractable decreases, and demand from developing nations, such as China, increases, the depletion of oil promises to occur with significant increases in price.

1.2 Motivation for low carbon fuel

In addition to oil's dwindling reserves, the substance also is at fault for two large environmental problems. Combustion of oil contributes to rises in the concentration of atmospheric carbon dioxide levels. The atmospheric concentration of CO_2 has risen from 280 ppm at pre-industrial levels to a current level of 401 ppm (National Oceanic & Atmospheric Administration, 2014). This is troubling news as a safe concentration of atmospheric carbon dioxide is 350 ppm (Hansen, 2008). Global air and ocean temperatures have increased 0.8 ºC since the early 20^{th} century, with more temperature increases to come (Board on Atmospheric Sciences and Climate (BASC); Division on Earth and Life Studies (DELS); National Research Council, 2011).

Combustion of petroleum has also contributed to ocean acidification, as the increased CO_2 levels are absorbed in the ocean to form carbonic acid. Ocean surface pH has dropped from 8.25 during pre-industrial times to 8.14 currently. This drop corresponds to a 30% increase in the concentration of H^+ ions (Jacobson, 2005). The decrease of ocean pH has led to significantly altered ocean chemistry. These new conditions have held negative consequences for many plant and animal species (Bryne, 2011).

There are multiple drivers for production of a low carbon liquid fuel, such as biofuels from organic wastes or lignocellulosic energy crops. A primary motive is mitigation of the environmental problems previously described. There are also, however, several positive reasons to pursue a low carbon liquid fuel. First among these is the abundance of cellulose, a polymer of sugars that forms a structural component of plant cell walls. Cellulose is an abundant organic polymer, which can be produced without the use of expensive inputs such as fertilizer or irrigation. Additionally, development of a low carbon liquid fuel would allow for fueling of machines incapable of being electrified in an environmentally benign way; electric storage in batteries is infeasible for extremely long-range vehicles such as boats and passenger or cargo airplanes. Thus there are numerous reasons for developing a carbon neutral liquid fuel—both for mitigation of negative consequences and the realization of positive ones. However, an economically feasible low carbon liquid fuel has yet to be developed.

1.3 Potential scale of biofuels

To understand the potential of biofuels to address policy goals and sustainability concerns, a number of research groups have recently been quantifying the possible scale of biofuels potential and use. A 2010 study concluded that 30% of the United States' transportation energy needs could be meet by biofuels, without decreasing domestic food production or agricultural exports (Dale, Bals, Kim, & and Eranki, 2010). This amount of substitution of biofuels is dependent upon the use of marginal land to grow crops for biofuels. With synergistic crop rotations to add energy crops during fallow periods on existing agricultural cropland, it is possible that biofuel may replace even more of the transportation energy requirements.

1.4 Differences between lignocellulosic and conventional biofuels

Lignocellulosic biofuels, the area this thesis explores, differ from conventional sugar and starch biofuels in significant ways. Lignocellulosic biofuels are more difficult to prepare than those made from starch or sugar. Production of biofuel from sugar crops involves only a single step: fermentation. Starch crops require two steps—saccharification, to break the long starch polymer into short glucose monomers, and fermentation. Lignocellulosic biofuels are the most difficult to produce biochemically[1], requiring three steps: pretreatment, a much more complex enzymatic hydrolysis than for starch to produce sugars, and finally the eventual fermentation. The pretreatment is necessary because lignin tightly binds the clusters of cellulose, rendering them inaccessible to bacteria. Enzymatic hydrolysis serves the same function for lignocellulose that saccharification does for starch—to break the long carbohydrate chain into sugar monomers that are digestible to bacteria, but requires much more time and three cellulase enzymes instead of one, amylase, for starch.

[1] There are multiple approaches; biochemical is one that has been developed. Thermochemical is an alternative and

Lignocellulosic biofuels further differ from sugar and starch biofuels in that they are produced from crops that are not used for food. This has the advantage of reducing the potential for conflict between food and fuel. Additionally, lignocellulosic biomass may be grown on marginal land, and some energy crops can grow without fertilizer. This increases its attractiveness for processing to biofuels.

Lignocellulose is a fibrous material forming the cell walls of plants. The material is composed of three substances:

- Cellulose, which consists of glucose polymers held together rigidly as bundles of fibers. The cellulose accounts for roughly 40 percent of the material of lignocellulose, by weight.
- Hemicellulose, which consists of shorter polymers of five and six carbon sugars, acts to hold cellulose bundles together with one another. Hemicellulose accounts for roughly 25 percent of the material of lignocellulose, by weight.
- Lignin, a polymer of propyl-phenol, is embedded in and bound to hemicellulose. It accounts for the rigidity of lignocellulose and takes up about 20 percent weight of the mass.

1.5 Biofuel production methods

Production methods for biofuel are organized into two categories: thermochemical and biological. Thermochemical conversion consists of heating biomass under reduced oxygen conditions. Operating conditions would normally fall in the range of 700 K to 1500K (Demirbaş, 2001). Pyrolysis and gasification are two types of thermochemical conversion, which each yield different products. Pyrolysis produces bio-oil, a mixture of organic compounds that has a chemical formula of $CH_{1.9}O_{0.7}$ (Ringer, Putsche, & and Scahill, 2006). Gasification produces syngas, a gas mix composed of carbon monoxide, hydrogen, and carbon dioxide. A hybrid system of gasification and biochemical conversion may be the most efficient route for production.

Biological conversion comes in two forms. Biomass may either be converted by anaerobic digestion or fermentation. Anaerobic digestion produces methane and carbon dioxide as products of digestion under low oxygen conditions. Fermentation has been developed for large-scale conversion of starch and sugar crops to ethanol, or other liquid fuel. First, biomass is ground and undergoes saccharification before fermentation to a transportation fuel, such as ethanol. Biological conversion is much more difficult for lignocellulose than starch or sugar crops. Both pretreatment and addition of enzymes are necessary in biological conversion of lignocellulosic material.

Biological conversion can further be classified according to the intermediate molecule that is produced and then converted to fuel. The type of intermediate molecule, either sugars or carboxylic acids, defined a "platform" from which various fuels and chemicals can be produced.

In the sugar platform, microorganisms convert digestible sugars from polysaccharide chains to produce fuel. Notably, most microorganisms cannot process hexose and pentose sugars at the same time, so biomass with a large proportion of glucose, cellulose, or starch is preferable. Furthermore, for processing any crop that is not a sugar crop, use of expensive enzymes and sterile conditions is necessary to break the polysaccharides into monomers that are digestible by the microorganisms.

The carboxylate platform converts complex biomass feedstocks into mixed carboxylic acids. This platform proceeds without the addition of expensive enzymes. A mixed culture of microorganisms hydrolyses the material and ferments it. One example of the carboxylate platform is the MixAlco process.

1.6 Method for cellulosic ethanol production

Current cellulosic biofuel production begins with pretreatment of the raw feedstock to break up the layer of lignin sheathing the cellulose. This pretreatment is necessary for any of the biological methods of biofuel production. The major current methods of lignocellulosic pretreatment are: steam explosion, chemical hydrolysis, and ammonia fiber expansion (Kumar, Barrett, Delwiche, & Stroeve,

2009). Steam Explosion, the most widely used pretreatment method, splits apart cellulose and hemicellulose from lignin, increasing its digestibility. Chemical hydrolysis accomplishes the pretreatment of the stream explosion method, but by use of either acid or base to break apart lignin, hemicellulose, and cellulose. Alkaline hydrolysis has several advantages over acid hydrolysis, as it degrades less of the sugar in the starting biomass, as well as offers potential for recovery of the caustic salts. Both high and low temperature methods of pretreatment are available for the two types of chemical hydrolysis. The two temperatures are for use with different operating conditions, such as batch versus continuous flow or a long versus short run time. Ammonia Fiber Expansion (AFEX) pretreatment acts much in the way that Steam Explosion does, except liquid ammonia is the substance used, instead of water. AFEX is most effective when used on biomass with a relatively low amount of lignin. The pretreatment process transforms biomass into much more digestible forms for mixed cultures of microorganisms or for enzymatic hydrolysis.

After pretreatment has occurred, the next step is to hydrolyze the biomass and break up polysaccharide chains into shorter sugar monomers. Most fermentation microorganisms more easily digest individual sugar units. Hydrolysis is accomplished with either the addition of dilute acid or enzymes to the pretreated material. There has been much research effort put into reducing the cost of enzymes used in cellulose hydrolysis, although they are still $0.50 to $0.75 per gallon of biofuel (Kumar, Barrett, Delwiche, & Stroeve, 2009). An alternative route is conducting hydrolysis in-situ, by culturing organisms that produce cellulase in the vessel, with the biomass. These hydrolysis methods make the production of biofuel expensive. Not only are the enzymes expensive, but when enzymes and pure cultures are used, expensive sterile operating conditions are required.

Fermentation follows hydrolysis. The traditional microorganism of choice when fermenting hydrolyzed material to fuel is baker's yeast, *Saccharomyces cerevisiae*. Recently however, engineered organisms have given promising results; *Zymomonas mobilis* and *Escherichia coli* are two of the best candidates (Jeffries & Jin, 2004).

1.7 Biofuel production by anaerobic digestion

Anaerobic digestion degrades organic materials under oxygen free conditions by microbial activity to produce biogas (a mixture of carbon dioxide and methane) and microbial biomass (NNFCC and The Andersons Centre, 2011). A variety of microorganisms behave synergistically to digest the material; bacteria, protozoa, and fungi produce the necessary hydrolytic enzymes. Compost, rumen, and swamps are al common locations for these microbial systems (Aiello-Mazzarri, Coward-Kelly, Agbogbo, & Holtzapple, 2005).

There are three distinct parts to anaerobic digestion, distinctions which are also used to categorize the organisms present—hydrolysis, acidogenesis, and methanogenesis. First, hydrolytic bacteria use cellulase enzymes to break apart long cellulose chains into simple, component sugars. Other biomolecules such as hemicellulose, starches, and proteins also undergo enzymatic degradation in a similar fashion. Subsequently, acidogenic bacteria ferment simple sugars to carboxylic acids. Some of the carboxylic acids undergo conversion by acetogenic bacteria to hydrogen, carbon dioxide, and acetate. Methanogens can convert acetogenesis products to the two undesired products of methane and carbon dioxide.

1.8 Acidogenic digestion and the Carboxylate Platform

Acidogenic digestion is a truncated form of anaerobic digestion. The usual progression of materials to carbon dioxide and methane is halted before methanogenic bacteria can act. Overall, the process converts biomass into mixed volatile fatty acids ranging from two carbon to six carbon (Datta, 1981). This initial production of organic acids can either be part of two phase conversion of biomass to methane gas, or it can be part of an alternative path toward biofuels. The latter option is considered for this thesis.

One specific example of biofuel production via acidogenic digestion is the carboxylate platform. This platform is less well studied than other current platforms like sugar or syngas. Instead of fermenting

alcohol from sugars, the route ferments sugars as well as proteins and other biomass constituents to mixed organic acids, which in turn may be reduced to alcohols or alkanes. The route furthermore uses a mixed culture of microorganisms that hydrolyze and ferment the biomass as a consolidated process. The carboxylate platform, therefore, does not require the input of enzymes for the hydrolysis step or sterile conditions—thus improving process economics a great deal (Holtzapple & Granda, 2009).

The MixAlco process is a specific example of an integrated biofuel strategy that uses the carboxylate platform. The process digests pretreated lignocellulose by a mixed culture of microorganisms to produce carboxylic acids. The lignin left undigested is gasified to produce hydrogen to reduce the carboxylic acids to alcohols or alkanes. The carboxylate platform offers unique advantages over either the sugar or thermochemical platforms for biofuel production. The advantages are summarized below (cite MixAlco paper):

- It has demonstrated the highest alcohol yields in literature, as compared to the sugar platform and thermochemical conversion
- It is the most energy efficient way to convert lignocellulose to fuels
- Sterile operating conditions are not needed.

The MixAlco process also has the advantage of being able to concentrate the energy of biomass in a much more compact form, to allow for ease of transportation. Fermentation of the biomass to a batch of mixed acids concentrates the energy of the biomass into a form easy to transport.

Acidogenic digestion provides much promise for economical conversion of biomass to liquid fuel. The concentrated organic acid product of the initial digestion phase is of much smaller volume than the original, raw material and as such would facilitate economical transport to a centralized bio-refinery. Following such consolidation and transport to a processing facility, the acid products could be converted to fuels and chemicals (Holtzapple & Granda, 2009). Potentially this is a platform with versatile applications for the production of new, lignocellulosic biofuels as well as value added chemicals.

Acidogenic digestion proceeds through the initial stages of anaerobic digestion using a mixed culture of microorganisms. As such, sterile processing is not needed, nor is the addition of cellulytic enzymes. As a result, the initial stages of acidogenic digestion offer considerable economic advantages over the sugar platform for production of lignocellulosic biofuels.

1.9 Acidogenic digestion production specifics

As covered earlier, acidogenic digestion proceeds to acetogenic digestion if left uninterrupted. To use the carboxylic acids as a biofuel platform, acetogenesis and methane formation must be inhibited. Multiple methods to inhibit methanogenesis exist. In the MixAlco process, iodoform and bromoform, methane analogues, have been shown to inhibit methanogenesis (Bauchop, 1967). Furthermore, promellitic diimide (Martin & Macy, 1985) and 2-nitro propanol (Anderson, Callaway, Van Kessel, Jung, Edrington, & Nisbet, 2003) have been shown to inhibit methanogenesis. Control of pH is another method that may be used to inhibit methanogenesis. A pH of 6.8 to 7.5 is required for methanogenesis in a conventional anaerobic digester, as such the phase may be inhibited by reducing pH below this range (Borchardt, 1971).

A variety of factors will influence the progress of an acidogenic digestion process, the most important of which is pH. In acidogenic digestion, organic acids can accumulate due to absence of methanogenic microorganisms to consume the acids (Eastman & Ferguson, 1981). The hydrolytic and acidogenic phases can be inhibited by the resulting drop of pH. This finding is supported by multiple studies; pH and substrate concentration are two important factors that influence the acidogenesis of biomass (Domke et al. 2004; Hwang et al. 2001; Sanchez et al. 2005). Digestion pH can also affect the composition of the acid stream generated. At pH 4.0-5.9, propionic acid and hydrogen are the main products, whereas at pH 6.0-7.0 acetic, butyric, and i-butyric acid are the main products (Yu & Fang, 2003).

Mixed cultures of microorganisms are used in acidogenic digestion. Their complex nature usually leaves cultures undefined. The original sources for inoculum used in acidogenic digestion are anaerobic environments such as compost, rumen, or silage.

1.10 Motivation for research

Agricultural wastes, energy crops, and municipal sewage have all been feeds for acidogenic digestion, but lignocellulosic material has been not been extensively investigated (Demirer and Chen, 2005; Chanakya et al. 1992, Ghosh 1991, Ghosh et al. 1985, Borja et al 2003). Therefore, the behavior and function of digestions lignocellulosic digestions is less well characterized. This thesis characterizes acidogenic digestion of a lignocellulosic biomass—willow. The digest is performed to optimize pH, a critical variable in reactor performance, for maximum production of organic acids. Additionally, the change in composition of the biomass and reactor medium will both be studied so as to gain.

Chapter 2

Materials and Methods

2.1 Steam Explosion Pretreatment

Hybrid willow, *Salix spp.*, harvested at three years of age, was air-dried and ground to 1/8" before steam explosion pretreatment. The used device was an Advance-Bio SuPR-2G model hydrolyzer (Advance-Bio, Milford, OH). The device was operated at 190 ºC, residence time 90 seconds, and with a pressure of 165 psig.

2.2 Inoculum Preparation

The inoculate used to inject the bioreactors was prepared from three different sources. Compost, corn silage, and rumen from a cow were combined to provide a diverse population of anaerobic bacteria for the digestion. The compost used to make the inoculum was at a late stage of digestion and composed of a variety of organic materials. Solid and liquid samples from the bottom of silage piles were taken to contribute to the inoculum. The last addition was cow rumen fluid, collected and filtered through cheesecloth. The biological samples were put into phosphate buffer saline (PBS). This buffer, per liter of distilled water, contained 0.2 g KCl, 0.24 g KH_2PO_4, 8 g NaCl, and 1.44g Na_2HPO_4. The pH was adjusted to reach 7.4 with concentrated NaOH. The compost, silage, rumen fluid, and PBS were combined in a 3:1:1:8 ratio. The resulting mixture was processed in a blender, before being passed through a 90 µm filter and centrifuged. The solid fraction was used to inoculate the bioreactors. The reactors were inoculated at the rate of 1/30 dry matter inoculum/ dry matter biomass.

2.3 Willow Acidogenic Digestion

In the performed experiment, there were five treatment levels with pH as the independent variable. The pH levels 4.5, 5, 5.5, 6, and 6.5 were examined to determine which produced the highest levels of organic acids. The bioreactors used in this experiment were one-liter Oxitop brand bottles. These bottles were loaded with 193 g wet mass pretreated willow which contained 25.8 percent dry matter, 8.59 g wet mass inoculum which contained 19.4 percent dry matter, and 200 mL ultrapure water. A control treatment of 8.59 g wet mass inoculum only was included in the experimental design, and maintained at pH 6 for the duration of the experiment. Each pH treatment level was carried out in triplicate, but with no replication of the control experiment. In total, this summed to 16 bottles. The digestion was conducted for 18 days, in an oxygen free environment of three percent hydrogen and 97 percent nitrogen. Temperature was held at 28 ºC. Samples were agitated to assure representative sampling during withdrawal of reactor fluid for organic acid analysis, and also during regular pH adjustments.

2.4 pH Control

To maintain treatment levels at their respective pH's, pH control was needed. The pH was kept at a constant level by compensating for the production of acid by regular addition of concentrated 8 M NaOH. The pH was measured and adjusted every other day. The pH drift was less than 0.15 pH units across the two-day period. To ensure uniform pH throughout sample bottles, the contents were agitated before initial pH readings, during pH adjustment, and after completion of pH readings.

2.5 Ion Chromatography

All organic acid quantification was carried out on a Dionex ICS-3000 (Dionex Corporation, Sunnyvale, CA) ion chromatograph (IC). Samples for organic acids quantification were collected every

other day. Prior to IC analysis, samples were filtered through a 2.5 μm filter and diluted with ultrapure water. The calibration curve used to integrate peaks in organic acids measurement was constructed with four acid concentrations for each of 12 different acids. Standards were prepared with 25, 50, 100, and 200 mg/L of organic acid. Tartaric, malic, pyruvic, succinic, lactic, formic, acetic, propionic, isobutyric, butyric, valeric, and hexanoic acid were measured. Due to the presence of long chain carboxylic acids, the run time for elutions was set to 85 minutes.

2.6 Compositional Analysis

Both pretreated and post-digestion biomass was characterized by compositional analysis. The method used was that published by the National Renewable Energy Laboratory (NREL) intended for feedstocks (Sluiter & Sluiter, 2011). The major steps in compositional analysis include:

1) Determination of Total Solids
2) Determination of Ash
3) Determination of Extractives
4) Determination of Structural Carbohydrates and Lignin
5) Determination of Starch

All necessary calculations were performed using the NREL published compositional analysis and mass closure spreadsheet. An Accelerated Solvent Extraction system (ASE), ion chromatograph, and spectrophotometer were used in the compositional analysis performed. The ASE system was a Dionex ASE 350, and the ion chromatograph Dionex ICS-3000 (Dionex Corporation, Sunnyvale, CA).

2.7 Calculations

Throughout the experiment, percent dry matter was calculated with the following equation.

$$Percent\ dry\ matter = \frac{W_d}{W_w} \times 100$$

In which,

$$W_d = oven\ dry\ weight \qquad W_w = wet\ weight\ of\ sample$$

Whenever the concentration of organic acids in a single sample is reported, the calculation proceeds as follows:

$$C_{OA} = C_{tartaric} + C_{malic} + C_{pyruvic} + C_{succinic} + C_{lactic} + C_{formic} + C_{acetic} + C_{propionic} + C_{isobutyric} + C_{butyric} + C_{valeric} + C_{hexanoic}$$

In which,

$$C_x = concentration\ of\ 'x'\ acid,\ in\ grams\ per\ liter$$

When the concentration of organic acids in a treatment level is reported, the calculation proceeds as follows:

$$C_{OA,treatment\ level} = \frac{\left(C_{OA_1} + C_2 + C_3\right)}{3}$$

In which,

$$C_{OOA_nA} = Organic\ acid\ concentration\ in\ individual\ sample\ 'n'$$

2.8 Statistical Methods

The dependence of acetic acid concentration at the time of maximum organic acid concentration on pH was assessed by one-Way ANOVA. Analysis was conducted with Microsoft® Excel® for Mac 2011. During analysis, alpha was taken to be 0.05.

Margin of error for measurements of organic acid concentration during the experiment was calculated based upon standard deviations. Reported error for concentrations of organic acid takes the form of:

$$Average\ concentration\ across\ treatment\ level \pm standard\ deviation\ of\ average$$

The standard deviation was calculated based on a sample basis, as opposed to a population. Sample size was taken to be three, for each of the triplicates in a treatment.

2.9 Constant ash calculation of biomass digested

As a part of yield calculations, it was necessary to determine the digested biomass fraction. These calculations were carried out based upon a constant ash assumption, as described in Ahn et al. 2005. Mass of ash was assumed not to change during the course of the experiment. This assumption, coupled with the initial and final concentrations of ash in solid samples, was used to determine the amount of biomass digested. The amount of biomass that was digested was in turn used to calculate the yield of organic acid per gram dry weight of digested biomass.

Chapter 3

Results and Discussion

3.1 Pretreatment

The pretreatment process produced a biomass with the following composition:[2]

Table 1 Willow composition after pretreatment

Component	Composition (%)
Ash	2.9
Extractives	6.8
Lignin	31.8
Glucan	30.1
Xylan	3.6
Galactan	0.5
Arabinin	0.0
Mannan	0.2
Uronic Acid	0.0
Acetate	5.6

This biomass was used as the input material for all digestions that were performed, and the ash concentration of this starting material was compared with the ash concentration in the final biomass residue to estimate the mass degradation using the constant ash assumption previously described.

3.2 Optimizing pH for the production of organic acids

Acidogenic digestion of willow yielded results varying from low acid production at pH 4.5 to high acid production at pH 6 and 6.5. The results of the experiment were in accordance with theory in many ways. The highest two pH treatment levels produced the greatest amounts of organic acid, thus

[2] During the analysis process, some mass was lost; components sum to 81.5%.

confirming theory about acid inhibition being less likely to occur at higher pH treatments. Similarly, the lowest two pH treatment levels produced the least amounts of organic acid, thus confirming theory that lower pH treatments are more likely to experience acid inhibition.

The treatment levels pH 6 and 6.5 gave statistically equivalent maximum organic acid concentrations of 29.42 ± 1.24 g/L and 29.75 ± 0.80 g/L. The two lowest pH treatments, 4.5 and 5, gave statistically equivalent maximum organic acid concentrations of 22.22 ± 1.32 g/L and 22.75 ± 3.71 g/L respectively. Falling in the range between was the middle pH, 5.5, yielding a maximum concentration of 25.85 ± 1.61 g/L organic acids. The lowest two pH's resulted in production of 34% less acid than the highest two pH's, and the middle pH produced 17.4% less. The change in organic acid concentration with time is presented in Figure 1.

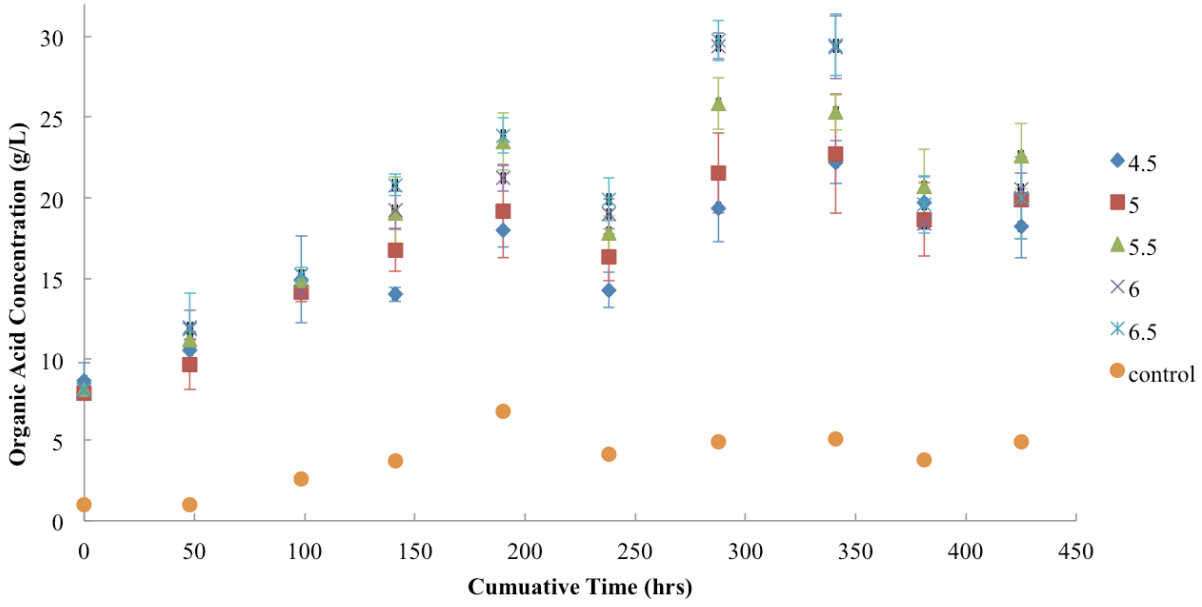

Figure 1 Organic acid concentration over time

The two highest pH treatments, 6 and 6.5, produced statistically equivalent amounts of acid at every sampling time except 190 hours. Acid concentrations of the two lowest pH treatments, 4.5 and 5,

were statistically equivalent for every sampling but 141 hours. The control was not carried out in replicate, and as such no error bars can be produced for it.

The maximum acid concentrations for pH treatments 6 and 6.5 were statistically equivalent. The upper pH value is close the range at which methanogenesis begins to occur, which typically starts at pH 6.8 (Borchardt, 1971). As there is no increase in yield that accompanies the increase in pH, the lower of the two treatment levels, pH 6, is the preferable point of operation.

In Figure 3.1 at 288 hours it is evident that there is a change in the rate of acid production. The changeover is first visible at 288 hours and likely indicates that inhibition is taking place. The type of inhibition is unknown; identification would require further testing. The buildup of organic acids (product inhibition), perhaps in combination with inhibitors produced during pretreatment such as furfural and/or HMF, may have caused the observed inhibition and stabilization of acid concentrations (Luo, Brink, & Blanch, 2002).

As digestion of the samples proceeded, differentiation among treatment levels gradually became apparent. Significant differences among the five pH treatments was only observable at and after the 4th sampling, t=141 hours. This suggests that pH may have a limited effect on productivity while organic acids are still low in concentration or there are still large amounts of simple sugars available (Kumar, Barrett, Delwiche, & Stroeve, 2009). Further testing is necessary to determine the mechanisms of this effect.

3.3 Profile of organic acids produced

It has been shown above that pH treatments gave differing yields of total carboxylic acid. In addition, these pH treatments also gave differing profiles of the different individual acids produced. Lower pH treatments resulted in higher absolute and relative amounts of hexanoic acid, while producing lower relative and absolute amounts of acetic acid. With higher pH treatments, the absolute amount of

butyric acid also increased. These comparisons were made when acid concentration was highest, at 288 hours. Nearly all differenced in yield were due to increased acetic acid and butyric acid production. Higher pH treatments showed greater amounts of these components.

The major acids produced were lactic, acetic, propionic, butyric, and hexanoic acid, and these five acids combined represented more than 95 percent of the total mass of acids present for all pH treatments. Formic acid was the only other acid that made up more than 1% of the total acid profile. The major acids are depicted below in Figure 3.2. Tartaric, malic, pyruvic, isobutyric, isovaleric, 2-methyl-butyric, and valeric acid are not shown as together they account for less than one percent of the produced acids.

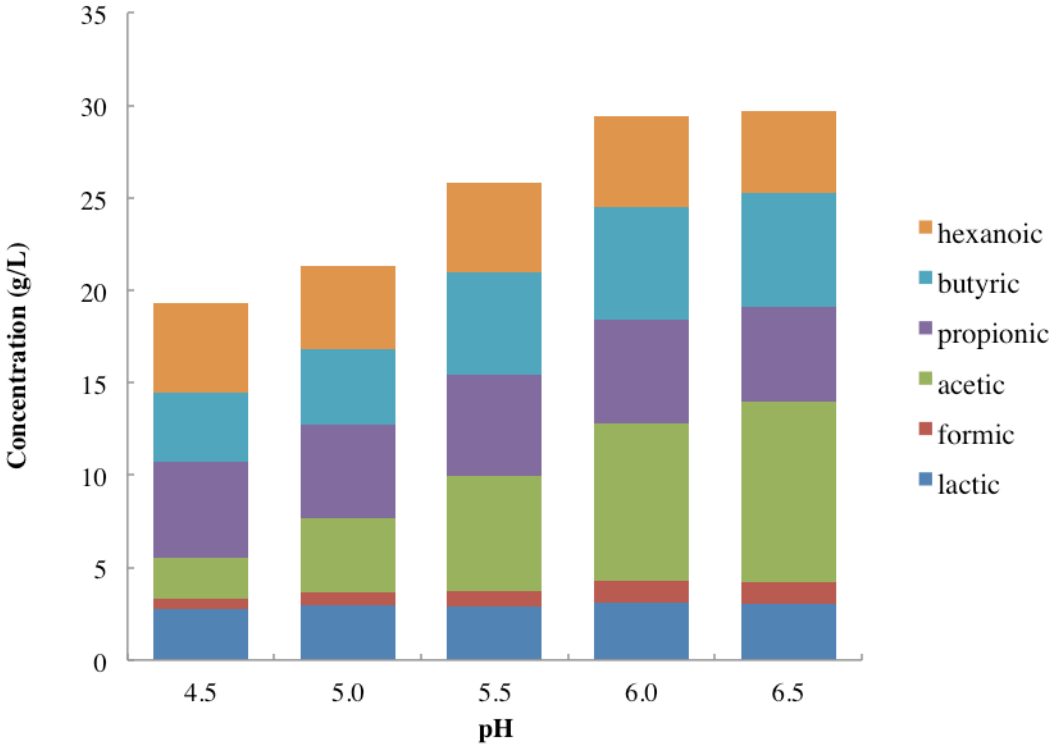

Figure 2 Effect of pH on acid profile at maximum organic acid concentration

Acetic acid was the dominant acid, and because it is an exellent intermediate molecule for downstream processing and increased over time, it was singled out for additional analysis. ANOVA testing was performed on the acetic acid concentration at 288 hours across treatment levels. The analysis

revealed that there is a relationship between the amount of acetic acid produced and pH. Results are summarized below in Table 2. Alpha was taken to be 5.0%.

Table 2 ANOVA table for acetic acid concentration

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p-level*	*F crit*
Between Groups	116,680,125.13514	4	29,170,031.28379	27.21979	0.00002	3.47805
Within Groups	10,716,480.23447	10	1,071,648.02345			
Total	127,396,605.36962	14				

The acid profiles for treatment levels 5.5, 6.0, and 6.5 were examined over time to better understand the behavior of the digestion. Results are summarized in Figure 3. The profile shifted to longer chain carboxylic acids as time progressed. More hexanoic and butyric acid were present as the digestion continued. This result is compatible with existing theory; long chain organic acids, known secondary metabolites, presented themselves late in the digestion.

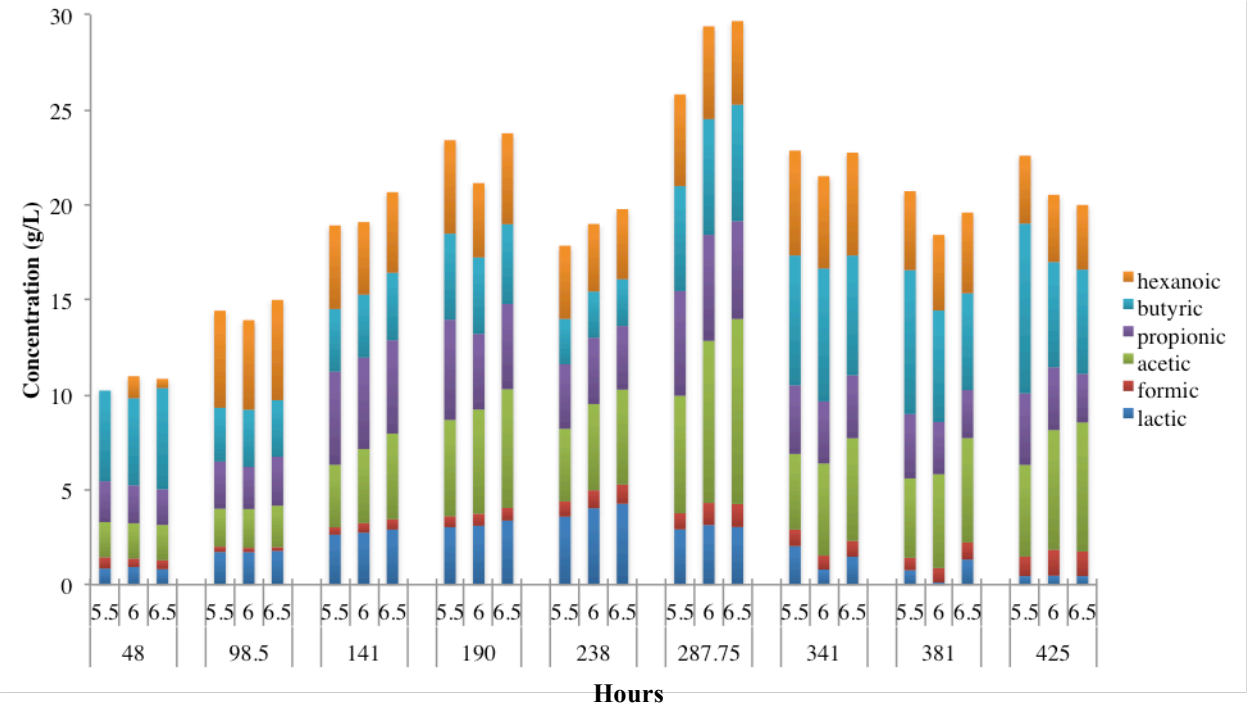

Figure 3 Acid profiles over time for pH 5.5, 6.0, and 6.5

3.4 Compositional Analysis

Compositional analysis performed on pre-treated and post-digestion biomass illuminated what changes took place during the digestion. The lowest pH level was not analyzed because analytical limitations only allowed for five samples, including the original biomass, to be completely analyzed. Composition results of the four higher pH treatment levels, pH 5 to 6.5, as well as the initial pretreated material are summarized below. Percent composition has been left out for uronic acid, mannan, arabinin, and galactan as they collectively account for less than two percent of any sample.

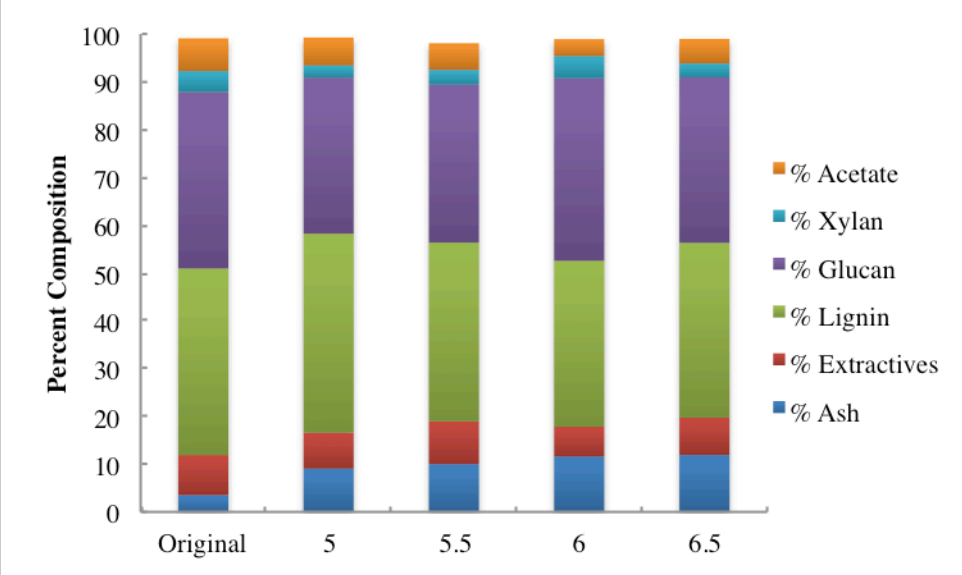

Figure 4 Compositional Analysis Results

Over the experiment, numerous changes in composition took place as some material was digested at comparatively quicker rates than others. In agreement with theoretical results, materials less conducive to digestion than other components displayed higher end fractions of the biomass. Likewise, materials more conducive to digestion dropped in concentration over the experiment. Note that the data in Figure 3.4 is on a concentration basis; to really understand these changes during digestion required conversion to a mass basis. While the solid residue after digestion was not directly measured, a constant ash assumption allows an estimate of the residual mass and, by difference, the mass digested.

Percent composition of ash increased for the material, from just over 3.5 percent to about 10 percent at the end of the digestion for each treatment level. Ash being indigestible, this result was expected. Percent glucan and xylan decreased over the course of the digestion, as could be expected by the material's easy digestibility. The glucan and xylan are fermented to acids over the course of the digestion.

Lignin displayed different changes during digestion; for some treatment levels it appeared to drop in percent composition, while for some increased. This result is contrary to theoretical predictions, as in acidogenic digestions the lignin has been shown to be largely indigestible (Datta, 1981). For clarification of the changes, or lack thereof, to lignin, additional replications of the compositional analysis is recommended. Likewise, changes in percent extractives of the biomass were inconclusive.

Other components represented so small a fraction of the tested material that they are left out of discussion of the compositional analysis results. The full results of compositional analysis are presented in Table 3.

Table 3 Compositional analysis results

Sample Treatment	% Ash	% Extractives	% Lignin	% Glucan	% Xylan	% Acetate	*% Galactan*	*% Arabinan*	*% Mannan*	*% Uronic acid*
Pretreated	3.5	8.4	39.0	36.9	4.4	6.9	*0.6*	*0.0*	*0.3*	*0.0*
Digested, pH 5, 425 hrs	9.1	7.5	41.6	32.7	2.5	5.8	*0.6*	*0.0*	*0.1*	*0.0*
Digested, pH 5.5 425 hrs	10.0	9.0	37.4	33.0	3.1	5.6	*0.5*	*0.0*	*1.5*	*0.0*
Digested, pH 6, 425 hrs	11.6	6.2	34.7	38.2	4.6	3.5	*0.7*	*0.0*	*0.4*	*0.0*
Digested, pH 6.5, 425 hrs	11.9	7.8	36.6	34.6	2.9	5.1	*0.7*	*0.0*	*0.4*	*0.0*

Mass closure for the tested treatment levels varied from low values of 78.6 percent to highs of 93.7 percent. Numerous factors contributed to these values not reaching 100 percent mass closure. Primarily, the compositional analysis procedure used was developed for raw, non-treated biomass feedstock, so applying it to pretreated biomass and especially pretreated, digested biomass could

negatively affect the quality of the results. One identifiable source of error is in the weighing and recording of the weight of post-digestion extractives. The organic acids that are post-digestion extractives are volatile and therefore able to evaporate before completion of proper weighing. Some of this volatile material may explain mass closures beneath 100 percent.

3.5 Yield

Organic acid yield was calculated to determine the highest performing pH treatment, where performance was defined by the highest yield of organic acids. Yield was calculated in two ways: on an initial biomass basis and on a digested biomass basis. The initial biomass basis measures the amount of acid that is produced per gram of starting material. The digested biomass basis provides a measure of the selectivity and efficiency of the conversion, as it measures how much of the material that was converted ends up as acid. In this thesis, yield on a digested biomass basis was calculated using a constant ash assumption. The results of yield calculations are organized below in Table 4 and plotted in Figure 5.

Table 4 Organic acid yields and digested biomass amounts

pH	Initial biomass (g)	Digested biomass* (g)	Produced acid (g)	Yield (g acid/ g dry ash free willow)		
				Starting biomass basis	Digested biomass basis	By acetic acid equivalents
4.5	193.1	27.9	13.54	0.28	0.49	1.12
5	193.0	29.8	14.83	0.31	0.50	1.18
5.5	193.1	32.6	17.73	0.37	0.54	1.31
6	192.9	36.7	21.31	0.44	0.58	1.41
6.5	193.0	36.1	21.59	0.45	0.60	1.36

* Calculated based on a constant ash assumption.

The highest yielding treatment was pH 6. This treatment level gave 0.43 g organic acids / g DW willow. This treatment level also produced the greatest yield on an acetic acid equivalent basis; 1.41 g acetic acid equivalent/ g DW willow was achieved.

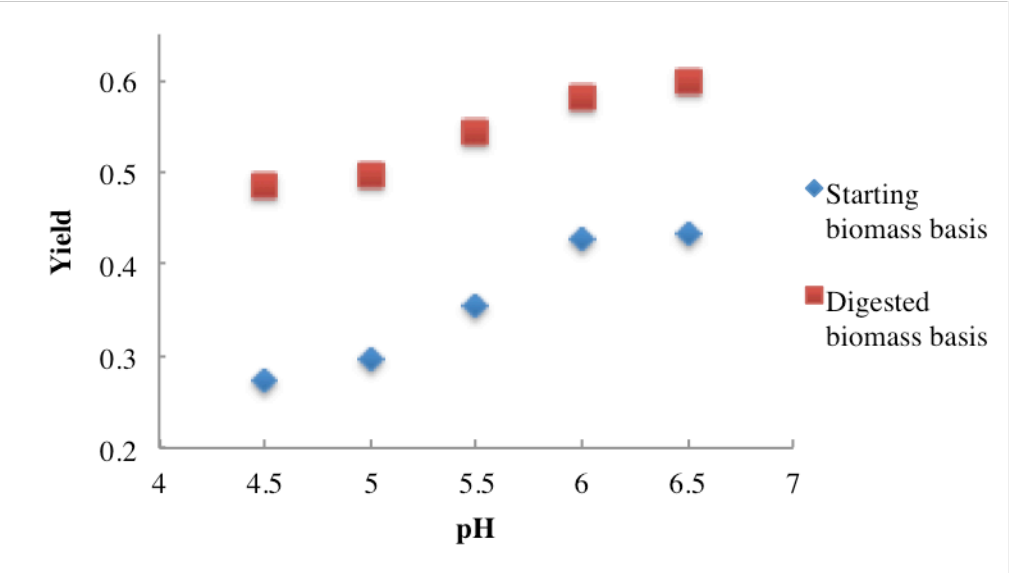

Figure 5 Organic acid yield versus pH

It is evident that yield increases with higher pH. Starting biomass and digested biomass basis calculations showed greater yields for higher pH treatments. This was consistent with theoretical understanding; higher pH levels have less inhibition and therefore allow greater acid production. Yields were increased greatly relative to the low levels at pH 4.5. With the high pH treatments of pH 6 and 6.5, the yield (starting biomass basis) was 1.6 times that of pH 4.5. Higher pH levels also produced more acid per gram DW willow starting material digested than low pH levels.

Yields plateaued at pH 6 and were not significantly increased further at 6.5. Based on this study's results, and on the large body of research associated with anaerobic digestion, it is unlikely that further increasing pH would increase acid production. From previous research, operating at or near pH 7 introduces almost certain methanogenesis to the digestion process, resulting in significant conversion of carboxylic acids to methane (Hwang et al. 2001).

The amount of biomass digested ranged from 15 to 20 percent. Higher digestion was observed with high pH treatment levels. Greater digestion might occur by use of in-situ acid removal, assuming the build-up of organic acids inhibited further digestion.

Chapter 4

Conclusion

Despite the substantial focus recently placed on developing liquid fuels that do not contribute to greenhouse gas concentrations, an economically viable process to produce biofuels has yet to be developed. Climate change still poses a threat as large as it ever has; however, mankind produces more carbon dioxide currently than at any point in its history (European Commission's Joint Research Centre, 2013). Climate change will affect everything from infrastructure stability to individuals' health and wellness. Scientists worldwide are in consensus that climate change is anthropogenic in nature (Intergovernmental Panel on Climate Change, 2013). Swift action on a global scale is necessary to avoid the most disastrous of climate change's consequences. Further motivation is provided by Earth's dwindling reserves of fossil fuel. With less than 50 years of conventional oil remaining, it is easy to understand the need for an economically viable low carbon liquid fuel not sourced from petroleum..

Efforts to produce low carbon biofuels have been in progress for some time, but have encountered numerous obstacles to development. Food crops are some of the simplest plants to convert into biofuel because of their high sugar content. However, using food crops for biofuel production leads to demand increases that raise food prices, with a deleterious effect on the general public. But with current technology, producing biofuels from crops not used for food involves considerable expense. Lignocellulosic biofuel is more difficult to produce than starch or sugar based biofuel. The additional enzymes required in traditional lignocellulosic biofuel production results in a cost structure that is not competitive with petroleum fuel. Lower cost technology to convert lignocellulose liquid fuel is required.

Acidogenic digestion of lignocellulosic biomass offers one potential strategy for a significant cost reduction. Acidogenic digestion's use of mixed bacterial cultures cuts production expenses in two ways: 1) a mixed culture does not require sterilization of the feedstock or reactor system, and 2) no costly enzymes are required. Instead, the organisms within the culture itself produce the enzymes that carry out

hydrolysis. This cost saving aspect of acidogenic digestion, coupled with high yields and potentially high stoichiometric efficiency as compared with conventional lignocellulose processing, make it a promising technology. Gaps in turning it into an economically viable technology include optimizing process conditions in the hydrolysis and acidogenesis phases to produce low cost biofuel intermediates, such as carboxylic acids. This thesis investigated the optimum pH, one key variable for the production of carboxylic acids from willow wood, a lignocellulosic biomass.

This pH optimization study produced multiple insights into the carboxylate platform. It was discovered that pH can be maintained below the levels at which methanogenesis typically occurs, without compromising yields of organic acid. Furthermore, this method produces high acid concentrations, 30 g/L at a maximum, with non-sterile conditions and without enzymes. Future research focusing on *in-situ* removal of acid products would be a logical next step to improve overall system performance.

Appendix A

Organic Acid Concentration Results

Bottles 1 through 3 are for pH treatment 4.5, 4 through 6 for pH treatment 5, 7 through 9 for pH treatment 5.5, 10 through 12 for pH treatment 6, 13 through 15 for pH treatment 6.5, and 16 for the control.

Sampling #	Date & Time	T_{cmltv} (hrs)	total [OA's] in g/L			
			Bottle #			
			1	2	3	Std Dev
0	7/10/13 20:00	0	9.90	7.81	8.31	1.09
1	7/12/13 20:00	48	9.96	10.35	11.35	0.72
2	7/14/13 22:30	98.5	12.42	17.81	14.62	2.71
3	7/16/13 17:00	141	14.08	13.57	14.43	0.43
4	7/18/13 18:00	190	18.23	16.86	18.87	1.03
5	7/20/13 18:00	238	13.55	13.77	15.53	1.09
6	7/22/13 19:45	287.75	17.64	18.76	21.75	2.12
7	7/25/13 1:00	341	20.91	22.20	23.54	1.32
8	7/26/13 17:00	381	19.60	18.14	21.35	1.60
9	7/28/13 13:00	425	16.02	19.01	19.65	1.94

total [OA's] in g/L							
Bottle #							
4	5	6	Std Dev	7	8	9	Std Dev
7.92	8.09	7.76	0.17	8.09	8.25	8.03	0.11
9.46	8.23	11.27	1.53	10.64	11.45	11.61	0.52
13.51	14.33	14.58	0.56	14.58	14.37	15.61	0.66
17.28	15.25	17.76	1.33	16.67	19.34	21.14	2.25
21.41	15.92	20.25	2.89	21.49	24.14	24.84	1.77
17.52	14.71	16.73	1.45	16.98	17.53	19.02	1.05
21.09	19.34	24.23	2.48	24.00	26.67	26.89	1.61
22.71	19.06	26.48	3.71	24.17	26.33	25.39	1.08
18.32	16.58	21.12	2.29	20.57	18.48	23.09	2.31
21.13	17.11	21.33	2.38	20.74	22.26	24.74	2.02

10	11	12	Std Dev	13	14	15	Std Dev	16
8.69	7.92	7.70	0.52	7.98	8.31	8.20	0.17	1.00
14.55	10.64	10.78	2.22	11.16	11.38	13.10	1.06	0.97
14.30	13.60	14.55	0.49	14.92	15.55	15.23	0.31	2.62
19.33	18.51	19.81	0.65	19.74	22.07	20.59	1.18	3.74
22.29	20.06	21.33	1.12	23.34	23.47	24.80	0.81	6.77
20.17	17.56	19.26	1.32	19.00	20.84	19.88	0.92	4.12
30.83	28.52	28.89	1.24	30.14	28.83	30.28	0.80	4.90
30.72	30.11	27.18	1.89	31.01	27.29	30.13	1.94	5.06
18.87	19.89	16.46	1.76	19.40	19.38	19.97	0.34	3.76
21.96	22.01	17.59	2.54	19.02	21.01	19.92	0.99	4.88

BIBLIOGRAPHY

Ahn, H., Richard, T., & Choi, H. (2010). The Influence of Ash Oxides on Reaction Stoichiometry Mass Balance Calculations during Biomass Transfer.

Aiello-Mazzarri, C., Coward-Kelly, G., Agbogbo, F., & Holtzapple, M. (2005). Conversion of municipal solid waste into carboxylic acids by anaerobic countercurrent fermentation: effect of using intermediate lime treatment. *Applied Biochemistry and Biotechnology* , 79-94.

Anderson, R. C., Callaway, T. R., Van Kessel, J. A., Jung, Y. S., Edrington, T. S., & Nisbet, D. J. (2003). Effect of select nitrocompounds on ruminal fermentation; an initial look at their potential to reduce economic and environmental costs associated with ruminal methanogenesis. *Bioresource technology* , 59-63.

Bauchop, T. (1967). Inhibition of Rumen Methanogenesis by Methane Analogues. *Journal of Bacteriology* , 171-175.

Bentley, R. (2002). Global oil & gas depletion: an overview. *Energy Policy* , 189–205.

Board on Atmospheric Sciences and Climate (BASC); Division on Earth and Life Studies (DELS); National Research Council. (2011). *America's Climate Choices.* Washington, D.C.: National Academies.

Borchardt, J. (1971). Biological waste treatment using rotating discs. *Biotechnology Bioengineering Symposium* , 34-39.

Borja, R., Rinc, B., Raposo, F., Alba, J., & Mart, A. (2003). Kinetics of mesophilic anaerobic digestion of the two-phase olive mill solid waste. *Biochemical engineering journal* , 139-145.

Bryne, M. (2011). Impact of Ocean Warming and Ocean Acidification on Marine Invertebrate Life History Stages: Vulnerability and Potential for Persistence in a Changing Ocean. In R. R. N. Gibson,

R. J. Atkinson, & J. D. Gordon (Eds.), *Oceanography and Marine Biology: An Annual Review.* Boca Raton, Florida: CRC Press.

Chanakya, H., Borgaonkar, S., Rajan, M., & Wahi, M. (1992). Two-phase anaerobic digestion of water hyacinth or urban garbage. *Bioresource technology* , 123-131.

Dale, B. E., Bals, B. D., Kim, S., & and Eranki, P. (2010). Biofuels Done Right: Land Efficient Animal Feeds Enable Large Environmental and Energy Benefits . *Environmental Science & Technology* , 8385–8389.

Datta, R. (1981). Acidogenic fermentation of corn stover . *Biotechnology and Bioengineering* , 61-77.

Demirbaş, A. (2001). Biomass resource facilities and biomass conversion processing for fuels and chemicals. *Energy Conversion and Management* , 1357–1378.

Demirer, G., & Chen, S. (2005). Two-phase anaerobic digestion of unscreened dairy manure. *Process Biochemistry* , 3542-3549.

Domke, S. B., Aiello-Mazzarri, C., & Holtzapple, M. T. (2004). Mixed acid fermentation of paper fines and industrial biosludge. *Bioresource technology* , 41-51.

Eastman, J. A., & Ferguson, J. F. (1981). Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *Journal (Water Pollution Control Federation)* , 352-366.

European Commission's Joint Research Centre. (2013). *Trends in global CO2 emissions: 2013 Report.* Amsterdam: PBL Publishers.

Ghosh, S. (1991). Pilot-scale demonstration of two-phase anaerobic digestion of activated sludge. *Water Science & Technology* , 1179-1188.

Ghosh, S., Ombregt, J., & Pipyn, P. (1985). Methane production from industrial wastes by two-phase anaerobic digestion. *Water research* , 1083-1088.

Hansen, J. e. (2008, April 7). Retrieved July 1, 2014, from http://www.columbia.edu/~jeh1/2008/TargetCO2_20080407.pdf

Holtzapple, M. T., & Granda, C. B. (2009). Carboxylate Platform: The MixAlco Process Part 1: Comparison of Three Biomass Conversion Platforms . *Applied Biochemistry and Biotechnology* , 525–536 .

Hwang, S., Lee, Y., & Yang, K. (2001). Maximization of acetic acid production in partial acidogenesis of swine wastewater. *Biotechnology and bioengineering* , 521-529.

Intergovernmental Panel on Climate Change. (2013). *Climate Change 2013: The Physical Science Basis.* Geneva: UNEP.

Jacobson, M. Z. (2005). Studying ocean acidification with conservative, stable numerical schemes for nonequilibrium air-ocean exchange and ocean equilibrium chemistry. *Journal of Geophysical Research: Atmospheres* , 110.

Jeffries, T. W., & Jin, Y.-S. (2004). Metabolic engineering for improved fermentation of pentoses by yeasts. *Applied Microbiology and Biotechnology* , 495-509.

Kumar, P., Barrett, D. M., Delwiche, M. J., & Stroeve, P. (2009). Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. *Industrial & Engineering Chemistry and Research* , 3713–3729.

Luo, C., Brink, D. L., & Blanch, H. W. (2002). Identification of potential fermentation inhibitors in conversion of hybrid poplar hydrolyzate to ethanol. *Biomass and Bioenergy* , 125–138.

Martin, S. A., & Macy, J. (1985). Effects of monensin, pyromellitic diimide and 2-bromoethanesulfonic acid on rumen fermentation in vitro. *Journal of Animal Science* , 544.

National Oceanic & Atmospheric Administration. (2014, July 7). *ESRL*. Retrieved July 16, 2014, from NOAA: http://www.esrl.noaa.gov/gmd/ccgg/trends/

NNFCC and The Andersons Centre. (2011). *NNFCC Renewable Fuels and Energy Factsheet: Anaerobic Digestion.*

Ringer, M., Putsche, V., & and Scahill, J. (2006). *Large-Scale Pyrolysis Oil Production: A Technology Assessment and Economic Analysis* . Battelle: National Renewable Energy Laboratory.

Sanchez, E., Borja, R., Travieso, L., Martin, A., & Colmenarejo, M. (2005). Effect of organic loading rate on the stability, operational parameters and performance of a secondary upflow anaerobic sludge bed reactor treating piggery waste . *Bioresource Technology* , 335-344.

Sluiter, J., & Sluiter, A. (2011, July). Summative Mass Closure. Golden, Colorado, USA.

U.S. Energy Information Administration. (2014, July 8). *Short-Term Energy Outlook.* Retrieved July 12, 2014, from U.S. Energy Information Administration: http://www.eia.gov/forecasts/steo/report/global_oil.cfm

Yu, H. Q., & Fang, H. H. (2003). Acidogenesis of gelatin-rich wastewater in an upflow anaerobic reactor: influence of pH and temperature. *Water research* , 55-66.

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