2015

Biochemical Conversion of Biomass into Butanol Using Clostridium acetobutylicum

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Biochemical Conversion of Biomass into Butanol Using *Clostridium acetobutylicum*

by

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With Honors

May 2015

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Abstract

Butanol is a potential transportation fuel which could eventually replace fossil-derived petroleum and transition the world towards a more sustainable future. This fuel can be produced by enzymatically hydrolyzing pretreated lignocellulosic biomass, and simultaneously fermenting the resultant sugars with specific bacterial strains. In this experiment switchgrass was chemically pretreated in a 75% (v/v) ethanol and 1% H_2SO_4 (1% v/v) solution and subjected to simultaneous saccharification and fermentation (SSF) with hydrolytic enzymes and *Clostridium acetobutylicum*. The pH control method for the SSF apparatus was varied between a 50 mM acetate buffer solution (HOAc), and the addition of calcium carbonate ($CaCO_3$) powder. Cellular products were quantified by HPLC analysis, and results were compared to identical procedures conducted on microcrystalline cellulose. The microcrystalline cellulose SSF procedures yielded 4.33 g/L (HOAc) and 4.27 g/L ($CaCO_3$) of butanol, with 0.037 g/L/min (HOAc) and 0.038 g/L/min ($CaCO_3$) butanol production rates. No butanol was produced from switchgrass, regardless of the pH control method. Butyric acid, the chemical precursor to butanol, was produced from switchgrass samples however, which suggests the final conversion step to butanol was most likely hindered. It is believed that the pH levels of the switchgrass SSF apparatuses were unsatisfactory, or that the acid-pretreated biomass released inhibitory compounds which prevented the biosynthesis of butanol.

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Acknowledgements

First, I would like to thank all those involved with my experience at Auburn University, where this research was carried out. Thank you to my research supervisor Dr. Maobing Tu for opening his research group to me, and for his guidance and support throughout my time at Auburn. Similarly, I would like to thank my graduate student supervisor Pixian Wang for his instruction and dedication to my laboratory experience and research project. Within the Tu research group, I would also like to thank Dr. Mi Li, Jing Li, and Xiaofei Wang for their support and mentorship. Lastly, I would like to especially show my gratitude for Dr. Sushil Adhikari for selecting me for this NSF-funded REU, and all of my REU fellows that I worked with this past summer.

In addition, it is essential that I acknowledge the wonderful support I have received from my faculty advisors here at the SUNY College of Environmental Science and Forestry. A special thanks to Dr. Stipanovic for acting as my thesis advisor, and guiding me through the process of constructing this thesis. Thank you to Dr. Christopher Nomura for reviewing this honors thesis, for the opportunity to continue a similar line of research in his research group, and for his excellent mentorship. I would also like to acknowledge Dr. William Shields and Dr. Neil Abrams for their guidance and help in securing my research position at Auburn University.

Lastly, I must recognize those closest to me that have supported my academic and research endeavors over my undergraduate career. To my parents Rod and Teresa, thank you for putting me through college, for flying oversized care packages to Auburn, and for standing behind the choices I've made. To my fellow students, Kristy Northrup, Julianna Kluger, Cameron Carter, Christopher Raber, Hank Ledeoux, Ashley Daniul, Liz Mummert, and all those in Music Society and the chemistry department here at ESF, thank you for acting as a wonderful, supportive family to me. Finally, my sincerest thanks go to my girlfriend Morgan Connolly, whose advice, care, encouragement, and love has truly impacted my academics, research, and life in amazing ways.

This research project was funded by the NSF Competitive Grant no. 1149940.

Introduction

To meet its energy needs, the United States primarily relies on nonrenewable fossil fuels; in 2011 for example, over four-fifths of the energy used in the US came from fossil fuels (EIA, 2012). The trend has not necessarily changed, as the US currently uses 18.45 million barrels of oil per day (EIA, 2014a). As the world's supply of fossil fuels dwindles however, attention has turned towards increasing production of bio-fuels from renewable biomass sources. Historically, the majority of bio-based transportation fuel in the US has come from corn-derived ethanol. In 2012, over 10 billion gallons of ethanol were produced from corn, but this only equated to ~10% of the total volume of gasoline consumed that year (EIA, 2014b; Xue et al, 2014). Compared to ethanol, butanol is a better-quality transportation fuel; butanol contains a higher energy density (~22% greater) and produces less physical wearing on existing automobile engines (BP, 2007; Wu et al., 2013). Further applications of butanol, especially in the synthesis of brake fluids, plasticizers, and detergents, are also under development (Yadav et al., 2014). Due to these desirable characteristics and uses, researchers have sought after ways to economically produce high yields of butanol from renewable biomass feedstocks.

One method of biological butanol production involves the anaerobic fermentation of sugars with various strains of anaerobic bacteria (Yadav et al., 2014). *Clostridium acetobutylicum* is one such bacterium, capable of fermenting sugars into n-butanol along with ethanol and acetone in a ratio of 6:1:3, respectively (Janssen et al., 1988). The fermentation process with *C. acetobutylicum* occurs in two distinct phases. In the first phase, acidogenesis occurs as sugars are converted into butyric and acetic acid. In the second phase, solventogenesis occurs as butyric acid and acetic acid are reincorporated into *C. acetobutylicum* and coverted to ABE products (i.e. acetone, butanol, and ethanol) (Wang et al., 2011) (Figure 1).

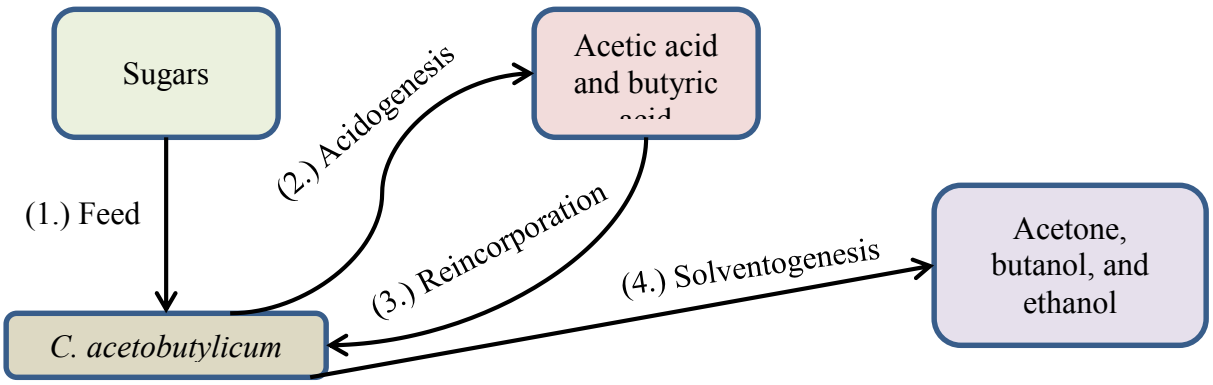

Figure 1. Flow diagram of ABE fementation process

C. aceotbutylicum can ferment both five- and six- carbon sugars, which can commonly be found in lignocellulosic biomass feedstocks, chemically linked together in long, polysaccharide cellulose and hemicellulose chains (Qureshi et al., 2008). Many butanol producing cultures, including *C. acetobutylicum*, cannot readily hydrolyze these chains into simple sugars however (Alonso et al., 2012; Rajagopalan et al., 2014). After physical and/or chemical pretreatment separates these polysaccharide chains from the tight, lignocellulosic matrix, additional enzymes are required to break them down into monomeric sugars for *C. acetobutylicum* to utilize (Haghighi et al., 2013). In order to create a more cost-effective process of obtaining butanol, hydrolysis and fermentation can be combined into a singular step, called "simultaneous saccharification and fermentation" (SSF). In this procedure, both hydrolysis and fermentation are carried out together in the same apparatus, allowing *C. acetobutylicum* to ferment monomeric sugars as they are produced (Qureshi et al., 2008).

As expected, different environmental factors within the SSF apparatus can affect the final butanol yield from *C. acetobutylicum* fermentation; pH for example, can have a significant effect on butanol production. During the acidogenic phase, the butyric acid and acetic acid produced by *C. acetobutylicum* reduces the environmental pH. If the acid concentration increases unchecked, an "acid-crash" may occur, where solventogenesis and cell growth will halt (Yang et al., 2013). In addition, formic acid, another byproduct of the acidogenic phase, may also bring about an "acid-crash" (Wang et al., 2011). Butyric acid however, although detrimental in large concentrations, is necessary for butanol production. Butyric acid re-enters the cells of *C. acetobutylicum* after its

production and acts as a chemical foundation for butanol synthesis. In fact, a baseline concentration of butyric acid is required for solventogenesis, and a direct correlation has been drawn between butyric acid concentration and butanol concentration at the beginning of this phase (Yang et al., 2013). Enzymatic conditions are another factor that can significantly affect butanol production. Enzymes directly supply *C. acetobutylicum* with monomeric sugars for use in ABE fermentation by hydrolytically cleaving cellulose and hemicellulose chains. Increasing the amount enzyme loading subsequently increases the amount of cellulose converted into monomeric sugars, and this effect is further amplified if the reaction is allowed to continue for multiple days (South et al., 1995). In addition, increasing the addition rate of enzymes at earlier stages of ABE fermentation can decrease the overall hydrolysis time, even with identical overall enzyme concentrations (Kim et al., 2008).

This research studied the effects of two different pH control methods (i.e. acetic acid and calcium carbonate) on the butanol yield from an SSF procedure with *C. acetobutylicum* using pretreated switchgrass as a feedstock. In addition, this study attempted to draw correlations between butyric acid production and enzymatic hydrolysis rates in relation to butanol production during these SSF procedures. We believed that the initial and overall production rate of butanol, as well as the final volume of butanol, would correlate directly to the initial enzymatic hydrolysis rate and the initial butyric acid production rate. Hydrolysis and fermentation results were compared to Avicel, a microcrystalline cellulose standard, which underwent identical experimental protocols.

Materials and Methods

1.) Biomass pretreatment

A quantity of dried switchgrass was soaked in a sealed, plastic container with a solution of 75% v/v ethanol and 1% v/v H_2SO_4. The biomass to organosolv solution ratio was 1:10. After soaking overnight, all contents were transferred into a Parr reactor, which was run at 150 ^oC for 1 hour. Once complete, the pretreated switchgrass was vacuum filtered and washed with hot water until neutral.

2.) Culturing *Clostridium acetobutylicum*

Microspores of *Clostridium acetobutylicum* ATCC-824 were obtained. A 0.2 mL sample was taken and briefly incubated in a hot water bath at 80 ^{o}C for 10 minutes. From this heat-shocked sample, 10 μL was inoculated onto a pre-made agar plate containing Reinforced Clostridial Media (RCM). The plate was sealed within an aeroPak anaerobic box and allowed to cultivate at 35 ^{o}C for two days. As the plate cultured, a 250 mL glass serum bottles was prepped for *C. acetobutylicum* inoculation; 1.9 g RCM and 50 mL Nano-pure water were added to the bottle, which was subsequently flushed with nitrogen gas for 10 minutes before being sealed. The bottle was then autoclaved at 120 ^{o}C for 15 minutes. Once cultivation was complete, a colony of *C. acetobutylicum* was inoculated into the autoclaved RCM bottle, which was finally incubated at 35 ^{o}C until its OD (optical density at 600nm) reached a point between 3 and 4.

3.) Subsequent Saccharification and Fermentation

Pretreated biomass samples for hydrolysis or SSF were prepared in a similar fashion to the RCM bottles. Eight glass serum bottles were prepared with 4.5 g pretreated switchgrass, 0.05 g yeast extract, 0.025 g L-(+)-cysteine hydrochloride monohydrate, 50 μL of an resazurin sodium salt oxygen indicating solution, and 1 mL of two mineral supplement solutions. Four bottles were designated to have an acetic acid buffer solution, while the other four would contain calcium carbonate powder. For the acid buffer bottles, 47 mL of Nano-pure water were added, as the buffer solution would be added after autoclaving. In the remaining four bottles, 0.25 g $CaCO_3$ powder was added, along with 48 mL of Nano-pure water. All bottles were flushed with nitrogen gas for 10 minutes, sealed, and autoclaved at 120 ^{o}C for 20 minutes. In order to compare results to a standard, another eight bottles were prepared in an identical fashion, with 2.73 g Avicel added instead of the 4.5 g pretreated switchgrass.

After autoclaving, 1 mL of the HOAc/NaOAc buffer solution containing 50 mmol/L of acetate was added to all bottles designated for the acid buffer. Following this, 5 mL of the inoculated RCM media with *C. acetobutylicum*, was added to half of the serum bottles. A small 0.5 mL sample was taken from each bottle at this point, marking zero hours. Finally, all bottles were inoculated with 0.44 mL of Novozyme

(Cellic CTec2) and put into a shaker at 35 °C and 200 RPM. Samples of identical volume were taken at regular intervals until hydrolysis or SSF had concluded.

4.) HPLC Analysis

All samples were centrifuged for 5 minutes at 1,200 RPM before HPLC preparation. A 100 µL portion of supernatant fluid was taken from each centrifuged sample and diluted 10-fold with Nano-pure water. From each diluted solution, 0.5 µL was filtered through a 0.2 µL VWR nylon filter into an HPLC vial. All samples were run through a Shimadzu HPLC equipped with a SIL-20AC HT autosampler/injector, LC-20AD liquid chromatograph, DGU-20A3 degasser, CBM-20A communications bus module, RID-10A refractive index detector, and CTO-20A column oven. The column (Aminex HPX-87P) was obtained from Bio-rad, and the guard column (MetaCarb 87H) was obtained from Varian. Each sample was run at 45 °C for 40 minutes with a 0.6 mL/min flow rate.

Results

All experimental results can be found within the figures below. Further information about the hydrolysis and SSF profiles of each sample set can be found respectively in Table 1 and Table 2 (*see appendix*). All graphs represent the average of two replicates for each experiment. Standard error bars were negligible, and not included.

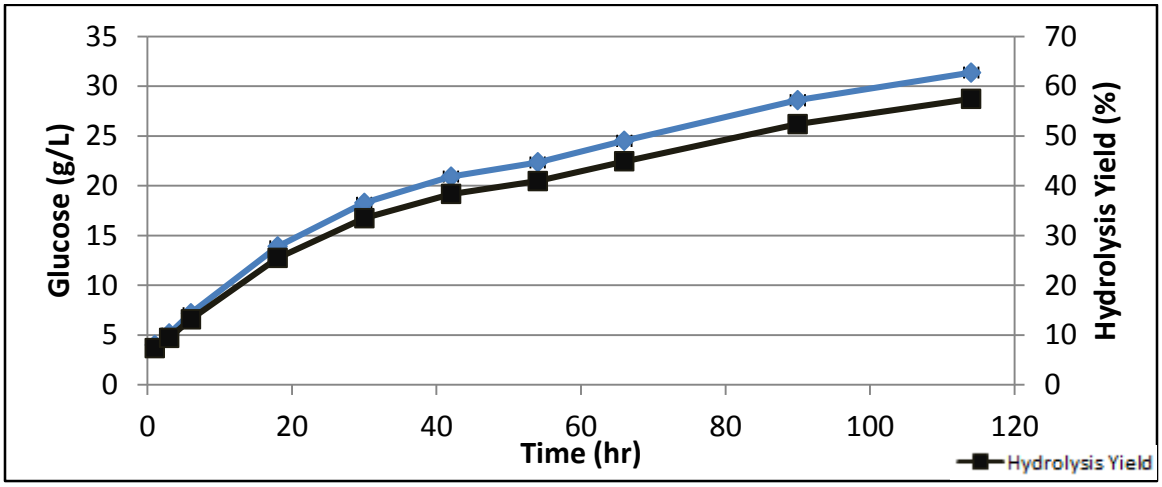

Figure 2. Hydrolysis profile for Avicel with HOAc buffer

The hydrolysis profile for the Avicel standards in the HOAc buffer (Figure 2) displayed a normal logarithmic curve, with the highest glucose production rate (0.570 g/L/min) occurring within the first three hours. An overall glucose yield of 31.4 g/L was achieved, which corresponds to an approximate 58% hydrolysis yield.

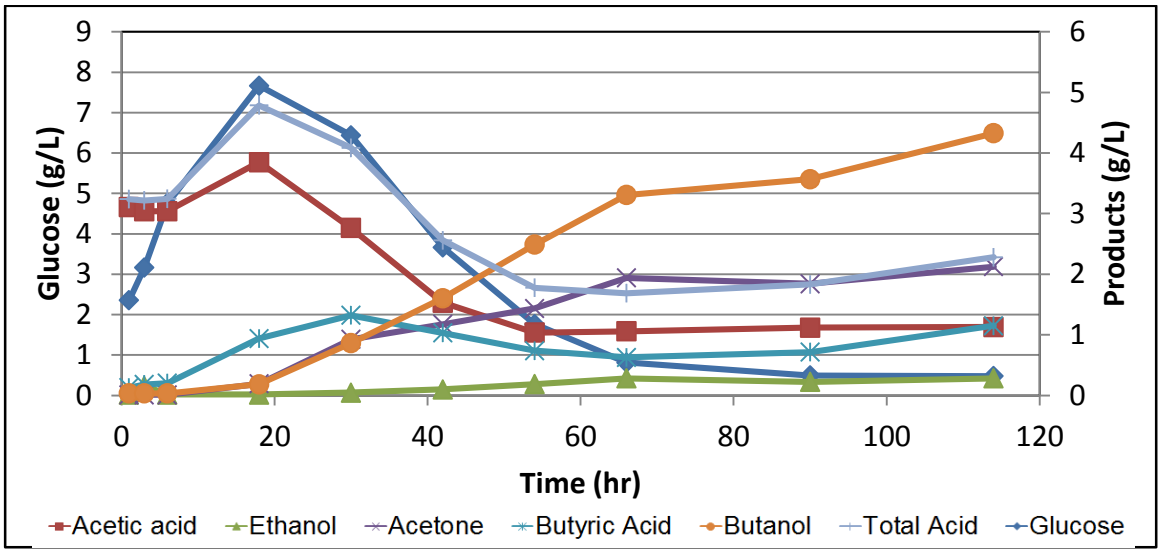

Figure 3. SSF profile for Avicel with HOAc buffer

All expected biosynthetic products (e.g., butyric acid, acetic acid, acetone, butanol, and ethanol) were detected in the SSF of Avicel with the HOAc buffer (Figure 3). In addition, glucose produced from hydrolysis was gradually consumed over time until its concentration was reduced to 0 g/L.The final butanol concentration was 4.33 g/L, which came from an average butanol production rate of 0.037 g/L/min.

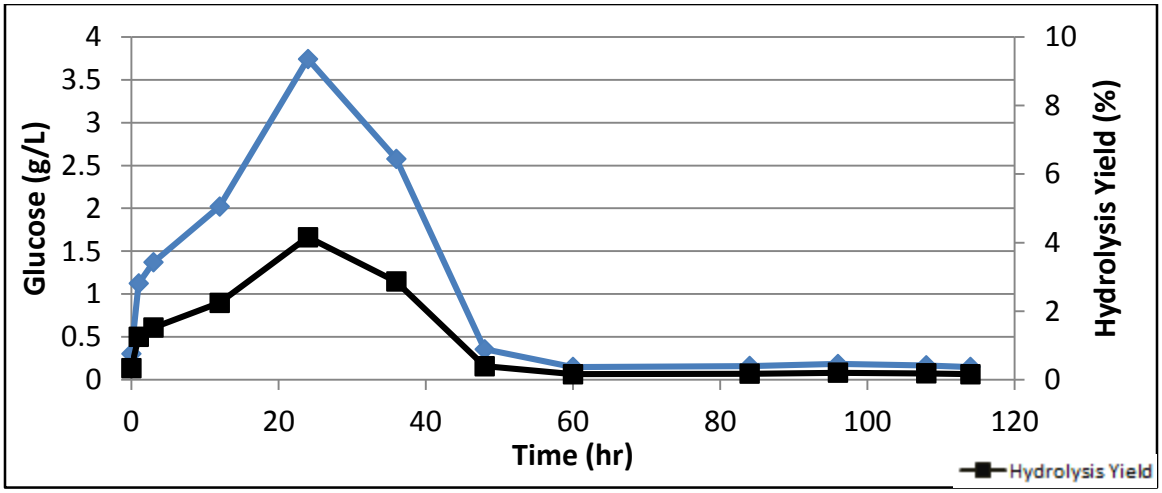

Figure 4. Hydrolysis profile for switchgrass with HOAc buffer

The hydrolysis profile of switchgass in HOAc (Figure 4) showed a gradual increase in glucose levels, followed by a sharp decline to roughly 0 g/L throughout the rest of the hydrolysis time. As a result, the hydrolysis yield for switchgrass – HOAc was effectively zero, as all the glucose was consumed after 50 hours.

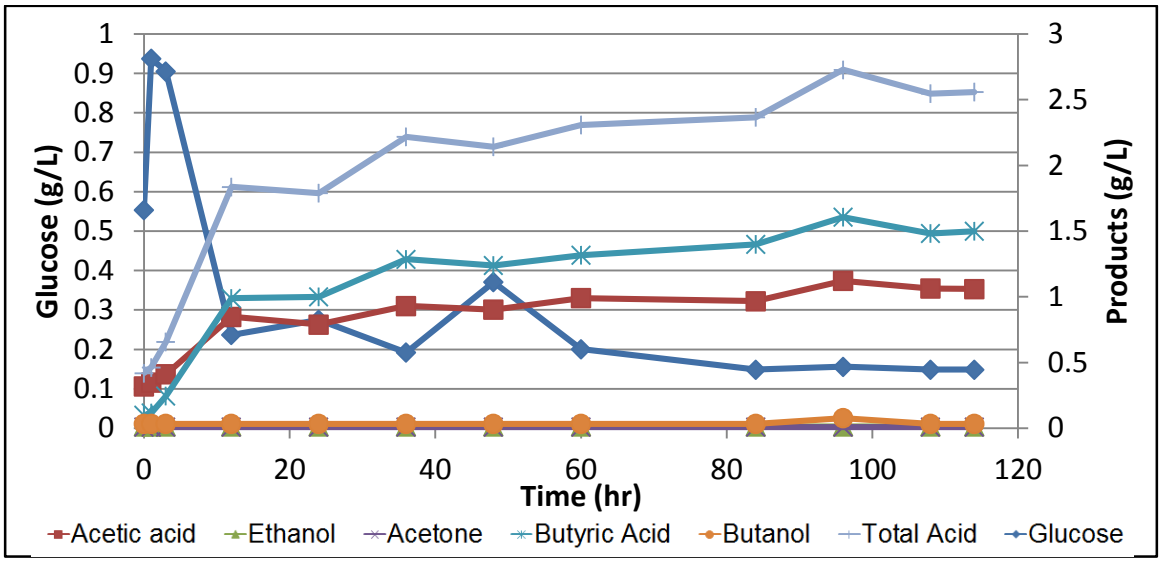

Figure 5. SSF profile of switchgrass with HOAc buffer

No butanol was produced from the SSF of switchgrass in the acetic acid buffer (Figure 5). Acidogenesis did occur however, and the concentrations of acetic and butyric acid gradually increased as glucose was steadily consumed. Ultimately, butyric acid reached a final concentration of 1.50 g/L after an initial production rate of 0.051 g/L/min.

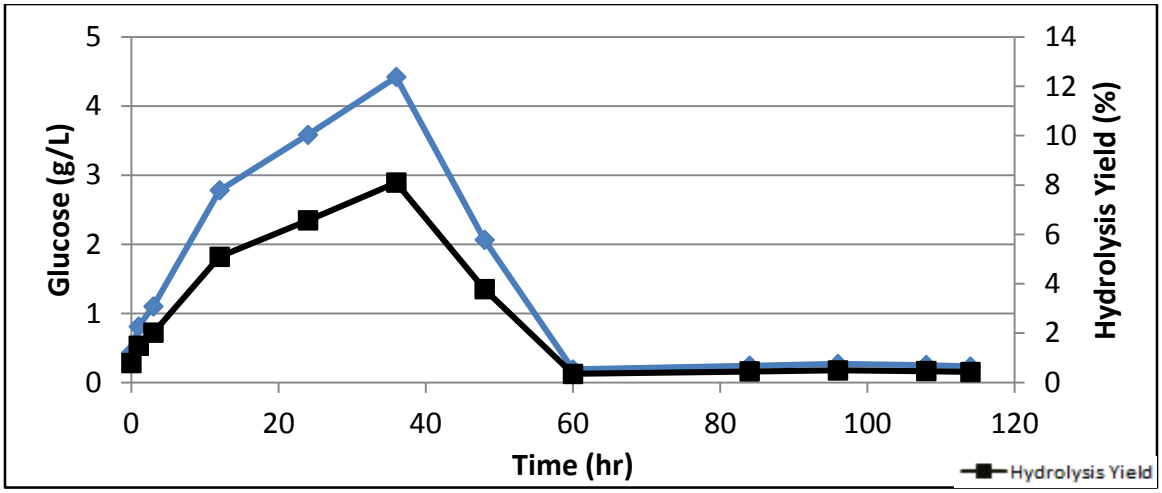

Figure 6. Hydrolysis profile for Avicel with $CaCO_3$ buffer

The hydrolysis profile of Avicel in the $CaCO_3$ buffer (Figure 6) also showed a sharp decline in glucose levels after a gradual increase. Again, the hydrolysis yield was effectively zero as all glucose appeared to be consumed.

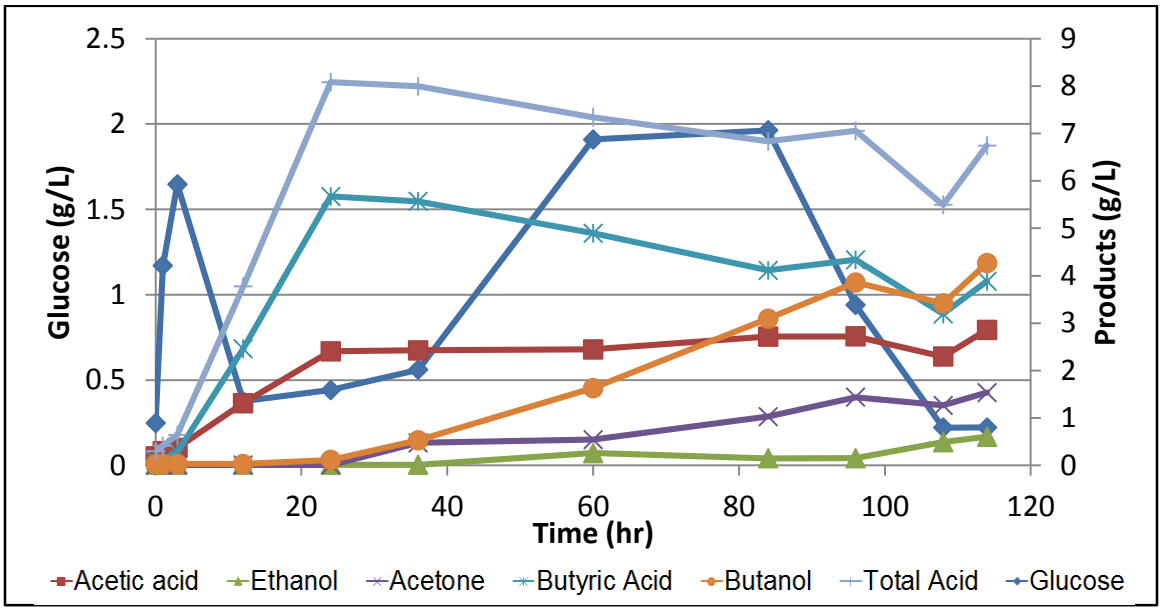

Figure 7. SSF profile for Avicel with $CaCO_3$ buffer

All expected biosynthetic products were detected in the SSF of Avicel with the $CaCO_3$ buffer (Figure 7). A final butanol concentration of 4.27 g/L was achieved, along with a 0.038 g/L/min butanol production rate. Between the 40th and 60th hour of SSF, glucose levels exceeded initial concentrations post-hydrolysis, and these high levels were maintained until after the 84th hour.

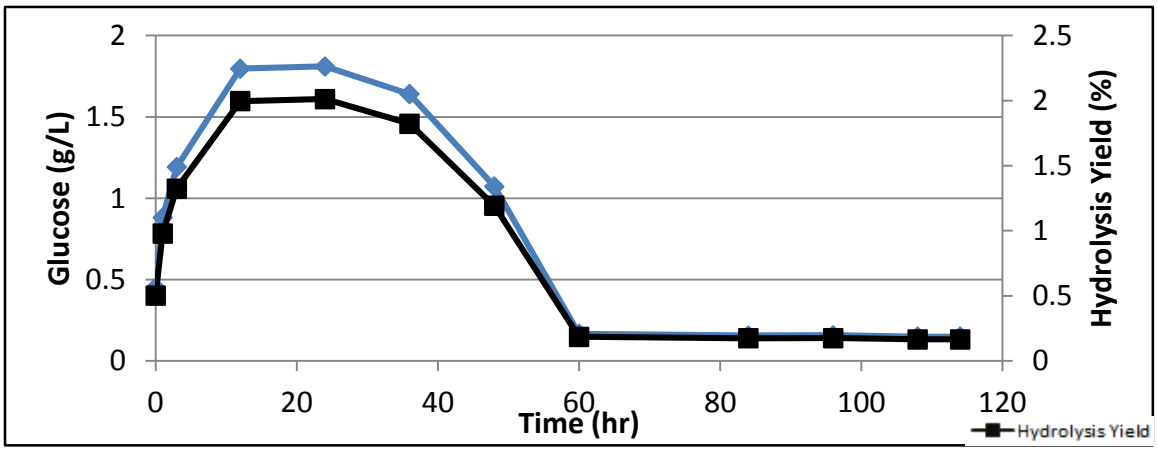

Figure 8. Hydrolysis profile for switchgrass with $CaCO_3$ buffer

After only the 12th hour into the hydrolysis of switchgrass in the $CaCO_3$ buffer (Figure 8), glucose production stalled and began to decrease. By the 60th hour, all glucose had been consumed, resulting in another 0% hydrolysis yield.

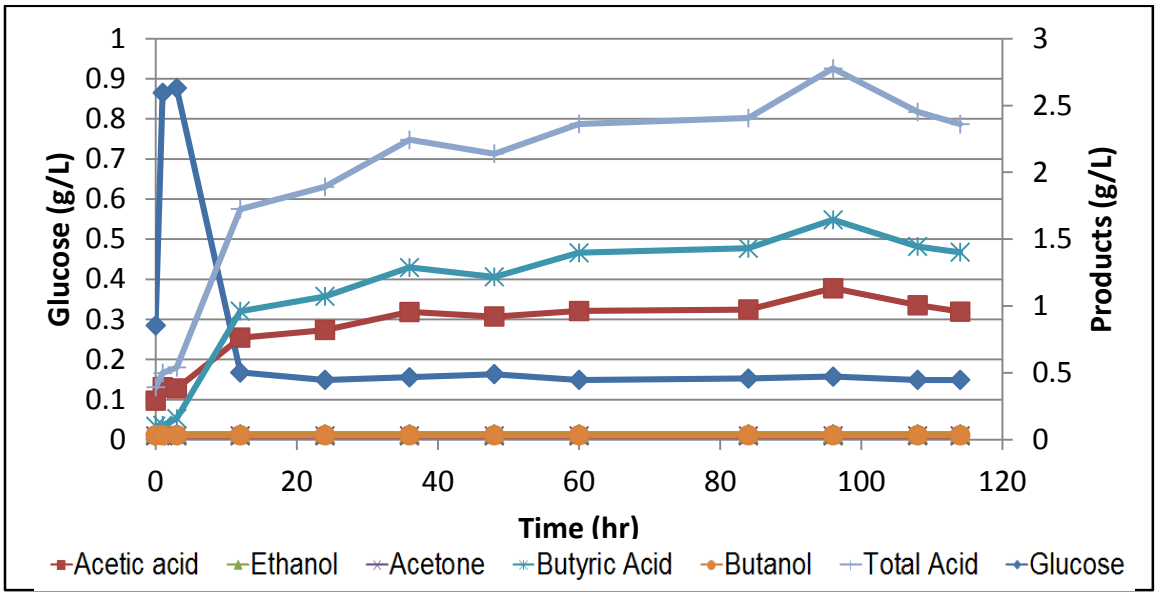

Figure 9. SSF profile for switchgrass with $CaCO_3$ buffer

Butanol was not generated in the SSF of switchgrass in the $CaCO_3$ buffer (Figure 9), but acidogenesis occurred. After an initial butyric acid production rate of 1.40 g/L/min, a final butyric acid concentration of 1.40 g/L was eventually reached. Glucose levels remained relatively constant as it was consumed to generate the intermediate acids.

Discussion

First, it is necessary to address the disparity between the hydrolysis profiles created from this experiment. Figure 1 shows a normal logarithmic hydrolysis curve, which should be the norm amongst all samples. Unfortunately, the glucose produced from the hydrolysis of switchgrass in HOAc or $CaCO_3$, as well as Avicel in $CaCO_3$, appeared to have been consumed by the end of the hydrolysis period. Bacterial growth (i.e. *Clostridium acetobutylicum*) was observed in these reaction vials around the 80th hour of all hydrolysis runs, which is most likely the culprit. Clearly, the experimental procedure will have to be modified to prevent contamination, especially when taking samples; a single heat-sterilized syringe can still hold some bacterial cells capable of

inoculating the hydrolysis media. A new syringe should be used for each sample, or the inside of the same syringe needle should be further sterilized in an alcohol solution. Due to this contamination issue, hydrolysis data across the four sample types cannot be readily compared until new samples are run in an identical manner. Furthermore, the hydrolysis data cannot be correlated to any data collected from the SSF trials for each sample type.

Unlike the hydrolysis data, the SSF results among all samples can be analyzed and compared. First, butanol was successfully produced from Avicel in either HOAc or $CaCO_3$. Both the final butanol yield and butanol production rate between these two trials were roughly the same, which could signify that the type of buffer solution does not have an immediate effect upon butanol production. Butyric acid production was also similar between the two sample types, although the final butyric acid yield for Avicel in $CaCO_3$ (3.88 g/L) was higher than Avicel in HOAc (1.15 g/L). Furthermore, glucose concentrations in the $CaCO_3$ trial rose to unexpectedly high levels midway through the SSF reaction, and these high glucose levels could have caused the higher butyric acid yield for Avicel in $CaCO_3$. The specific cause of this glucose spike is unknown, but all glucose was eventually consumed in both buffer solution trials for Avicel, signifying the efficient usage of glucose by *C. acetobutylicum*.

Although butanol was successful produced from Avicel standards, it is believed that the final concentrations could have been higher. The "anaerobic" jars used to carry out these SSF procedures could not be completely flushed on oxygen, as shown by a colored oxygen indicator. In order to increase butanol levels and prevent aerobic inhibition of *C. acetobutylicum*, procedural modifications must be made to further purge the jars of oxygen. Otherwise, all reactions should be carried out in an anaerobic chamber to prevent oxygen influx. Another possible hindrance to final butanol concentrations could have been the pH inside each anaerobic jar. If the pH somehow exceeded the effective buffering range of either solution, then a slight acid-crash may have occurred and impeded butanol production. To be sure, pH should also be measured along with intermediates and products during the SSF reactions.

In comparison, the SSF reactions for switchgrass in either buffer solution did not produce any butanol, ethanol, or acetone. Acidogenesis was apparent in the buildup of acetic acid and butyric acid, but the final transition to solventogenesis was not reached.

Final butyric acid concentrations for both trials were relatively similar, even after a much lower initial butyric acid production rate for switchgrass in $CaCO_3$ (0.02 g/L/min) than in HOAc (0.051 g/L/min). Either buffer solution appears to be suitable for carrying out an SSF reaction. The previously discussed issues involving the hindrance of butanol production in the Avicel reactions could have helped cause the lack of butanol production for the switchgrass samples. Surely, a semi-aerobic environment and a high pH could prevent *C. acetobutylicum* from beginning solventogenesis. In addition however, the lack of butanol production could have stemmed from original pretreatment method. Acid pretreatmenting biomass creates the risk of producing inhibitory compounds (e.g., furfural and hydroxymethyl furfural) upon hydrolysis (Haghighi et al., 2013). To further investigate this possibility, an alkali pretreatment should be performed, and the experiment should be repeated in a similar fashion. With an alkali pretreatment, the production of inhibitory compounds would be reduced. To support this theory, a portion of the hydrolysate should be subjected to analytical testing which could identify the presence of inhibitory compounds. Ultimately, the absence of solventogenesis could be a result of biomass recalcitrance; no biomass composition tests occurred before or after the pretreatment, so it is unknown whether any lignin was removed during the pretreatment. Had lignin not been removed during this process, then the hydrolysis and fermentation of the cellulose and hemicelluloses could be significantly hindered (Lim et al, 2013).

Conclusion

Overall, butanol was successfully produced from the simultaneous saccharification and fermentation of Avicel, but not chemically-pretreated switchgrass. Acidogenesis successfully occurred in the switchgrass samples, although solventogenesis did not. Useful information was still collected from this experiment, however. First, heat sterilization of the sample syringe was not effective enough to prevent contamination of the hydrolysis jars with *C. acetobutylicum,* so further sterilization must be taken into consideration. Secondly, both buffer solutions did not inhibit butyric acid or butanol production, so both HOAc and $CaCO_3$ could be considered appropriate buffers for these SSF reactions. Third, the acid-pretreatment of switchgrass could be inhibitory for butanol

production using *C. acetobutylicum*. A different pretreatment method, such as alkali or ionic liquid, should be considered as replacements.

References

Alonso, D. M., Wettstein, S. G., & Dumesic, J. A. (2012). Bimetallic catalysts for upgrading of biomass to fuels and chemicals. Chemical Society Reviews, 41, 8075-8098.

BP. (2007). *1-Butanol as a gasoline blending bio-component*. Retrieved from EPA website: http://www.epa.gov/oar/caaac/mstrs/March2007/Wolf.pdf

EIA (2014). *Countries - U.S. Energy Information Administration (EIA)*. Retrieved from http://www.eia.gov/countries/index.cfm?view=consumption#allcountries

EIA. (2014). *How much ethanol is in gasoline and how does it affect fuel economy?* Retrieved from http://www.eia.gov/tools/faqs/faq.cfm?id=27&t=4

EIA (2012). *Energy Perspectives: Fossil fuels dominate U.S. energy consumption - Today in Energy - U.S. Energy Information Administration (EIA)*. Retrieved from http://www.eia.gov/todayinenergy/detail.cfm?id=9210

Haghighi Mood, S., Hossein Golfeshan, A., Tabatabaei, M., Salehi Jouzani, G., Najafi, G. H., Gholami, M., & Ardjmand, M. (2013). Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. Renewable and Sustainable Energy Reviews, 27(0), 77

Janssen, P. J., Jones, W. A., Jones, D. T., & Woods, D. R. (1988). Molecular analysis and regulation of the glnA gene of the gram-positive anaerobe Clostridium acetobutylicum. Journal of Bacteriology, *170*(1), 400–408.

Kim, S., & Dale, B. (2008) Life cycle assessment of fuel ethanol derived from corn grain via dry milling. Bioresource Technology, 99(12), 5250–5260.

Lim, W., Kim, J., Kim, H., Choi, J., Choi, I., & Lee, J. (2013). Structural properties of pretreated biomass from different acid pretreatments and their effects on simultaneous saccharification and ethanol fermentation. Bioresource Technology, 139(0), 214-219.

Qureshi, N., Saha, B. C., Hector, R. E., Hughes, S. R., & Cotta, M. A. (2008). Butanol production from wheat straw by simultaneous saccharification and fermentation using Clostridium beijerinckii: Part I—Batch fermentation. Biomass & Bioenergy, *32*, 168-175.

Rajagopalan, G., He, J., & Yang, K. L. (2014). Direct fermentation of xylan by clostridium strain BOH3 for the production of butanol and hydrogen using optimized culture medium. Bioresource Technology, 154(0), 38-43.

Wang, S., Zhang, Y., Dong, H., Mao, S., Zhu, Y., Wang, R., … Li, Y. (2011). Formic Acid Triggers the "Acid Crash" of Acetone-Butanol-Ethanol Fermentation by Clostridium acetobutylicum. Applied and Environmental Microbiology, *77*(5), 1674–1680.

Wu, Y.-D., Xue, C., Chen, L.-J., & Bai, F.-W. (2013). Effect of zinc supplementation on acetone–butanol–ethanol fermentation by Clostridium acetobutylicum. Journal of Biotechnology, *165*(1), 18–21.

Xue, X., Pang, Y., & Landis, A. E. (2014). Evaluating agricultural management practices to improve the environmental footprint of corn-derived ethanol. Renewable Energy, 66, 454–460. doi:10.1016/j.renene.2013.12.026

Yadav, S., Rawat, G., Tripathi, P., & Saxena, R. K. (2014). A novel approach for biobutanol production by clostridium acetobutylicum using glycerol: A low cost substrate. Renewable Energy, 71(0), 37-42.

Yang, X., Tu, M., Xie, R., Adhikari, S., & Tong, Z. (2013). A comparison of three pH control methods for revealing effects of undissociated butyric acid on specific butanol production rate in batch fermentation of Clostridium acetobutylicum. AMB Express, *3*(1), 3.

Appendix

Table 1. Hydrolysis results for each experimental procedure

Hydrolysis Method	**Initial Hydrolysis Rate – Hours 1-3 (g/L/min)**	**Final Glucose Yield (g/L)**
Avicel - HOAc	0.570	31.365
SG - HOAc	0.322	0.165
Avicel – $CaCO_3$	0.212	0.426
SG – $CaCO_3$	0.234	0.165

Table 2. SSF results for each experimental procedure

SSF Method	**Initial Butyric Acid Production Rate – Hours 1-3 (g/L/min)**	**Final Butyric Acid Yield (g/L)**	**Butanol Production Rate (g/L/min)**	**Final Butanol Yield (g/L)**
Avicel - HOAc	0.066	1.149	0.037	4.325
SG - HOAc	0.051	1.500	-	-
Avicel – $CaCO_3$	0.062	3.884	0.038	4.265
SG – $CaCO_3$	0.020	1.403	-	-

Agar plate contents: 38 g/L RCM, 20 g/L agar

Mineral supplement 1: 10 g/100 mL $(NH_4)_2SO_4$, 3.75 g /100 mL KH_2PO_4, 3.75 g /100 mL K_2HPO_4

Mineral Supplement 2: 2g /100 mL $MgSO_4$, 0.05 g/100 mL $MnSO_4$, 0.08 g/100 mL $FeSO_4$

Oxygen indicator solution: 0.1 g resazurin sodium salt /100 mL water