

COUNTERCURRENT CONVERSION OF BIOMASS TO SUGARS

An Undergraduate Research Scholars Thesis

by

HEATHER BROOKS, JOHN DERNER and RUSSELL YANG

Submitted to Honors and Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by
Research Advisor:

Dr. Mark Holtzapple

May 2015

Major: Chemical Engineering

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	1
ABSTRACT	2
NOMENCLATURE	3
CHAPTER	
I INTRODUCTION	4
Objectives	4
II METHODOLOGY	6
III RESULTS	8
IV CONCLUSION	15
REFERENCES	16

ACKNOWLEDGMENTS

The writers of this thesis would like to thank Dr. Mark Holtzapple for giving us the opportunity to participate in research. We would also like to thank PhD candidates Sagar Lonkar and Chloe Liao along with other undergraduates who worked in the lab.

ABSTRACT

Countercurrent Conversion of Biomass to Sugars. (May 2015)

Heather Brooks, John Derner, and Russell Yang
Department of Chemical Engineering
Texas A&M University

Research Advisor: Dr. Mark Holtzapple
Department of Chemical Engineering

Our goal was to research and implement a countercurrent system to run enzymatic saccharification of biomass. The project provided clear results to show that this method is more efficient than the batch process that companies currently employ. Excess time, materials, and money are spent on the batch process because, until now, it has been the most efficient way to produce sugars needed in the food, chemical, and fuel industries. Due to the success of our project, we hope companies will utilize continuous countercurrent saccharification.

NOMENCLATURE

HPLC – High Performance Liquid Chromatography

CTec 3 – enzyme cellulase complex

HTec 3 – enzyme hemicellulose complex

BCA Assay- Bicinchoninic Acid Assay

CHAPTER I

INTRODUCTION

Little research has been done to test the efficiency of a complex countercurrent system. Simple, three-stage systems have been studied, but not to the extent as apply to large-scale production (Zentay 4). We worked to provide data that shows sugar production can be improved through a more complex countercurrent system. Countercurrent systems have been implemented for various biological systems (PubMed) and are effective in the body, but can also prove helpful in the food industry.

Objectives

Organic material can be processed to produce sugars and proteins. In our experiment, we used biomass from corn stover. Corn stover is the part of the corn plant left over after the ear is harvested. Corn stover is used as fertilizer in fields without deep topsoil, however, the topsoil is rich enough that the corn plant biodegrading in the field is not especially useful. It is cheap and readily available throughout the Midwest. Therefore, corn stover is very accessible for companies.

The corn stover we used was dried and broken up to be used as the biomass in this experiment. We broke up the biomass using a shock method pretreatment; raw biomass slurry was “shocked” by a controlled gas explosion, thereby breaking up the biomass and increasing its digestibility. The enzymes CTec 3 and HTec 3 were used to break down the biomass and turn it into sugar. Enzymes were added in different quantities and proportions to determine the most efficient

combination. These sugars can be useful in many different industries. For example, the sugar can be eaten by yeast to produce a form of single-cell protein used in pet and human foods alike. Our project seeks to optimize the process of using enzymes to hydrolyze cellulose and hemicellulose to glucose and xylose respectively.

CHAPTER II

METHODOLOGY

Most of our work took place in the laboratory. In the project, fresh solids and liquids were inserted at opposite ends of a 16-bottle system and flow in countercurrent. They exit at opposite ends as liquid product (sugars) and waste solids. The solid we used was shock-pretreated corn stover. We added biomass every 48 hours to Bottle 1 and transferred enough solids out to achieve a 90 g wet cake mass in the bottle. This procedure was repeated for Bottle 2 to Bottle 16. The solids exiting Bottle 16 were removed as waste. The amount of mass transferred from each bottle was the mass currently in the bottle, plus the mass to be added in, minus 90 g. This enabled the system to reach steady state while the enzymes had a chance to digest the mass. Conversely, we added deionized water and citrate buffer to Bottle 16 and transferred all liquids from each bottle down to Bottle 1. After centrifuging, all liquids coming out of Bottle 1 were removed from the system. This liquid was analyzed for glucose, xylose, lignin, and other possible plant byproducts. The most effective method was through an HPLC machine which used a column that was able to detect sugars.

Once all transfers were complete, enzymes were added to Bottle 4 and Bottles 1-16 were treated with antibiotics tetracycline and cyclohexamide to inhibit contaminant growth. The initial enzyme used was CTec 3 with a loading of 1mg/g of biomass. This particular enzyme was effective at digesting the biomass, but was specifically meant for breaking down cellulose to glucose. Our project sought to optimize both glucose and xylose output. Therefore, after the first steady state was reached, HTec 3 was added in addition to the CTec 3, with loading of 1mg/g of

biomass of both CTec 3 and HTec 3. HTec 3 enzyme targets hemicellulose, which is broken down into xylose.

CHAPTER III

RESULTS

The results of the project turned out similar to what we anticipated. The countercurrent process using enzymes HTec 3 and CTec 3 was three times more effective than the current batch process previously used to manufacture sugars. As enzymatic conversion accounts for a quarter of the total cost of ethanol production, this method will cut-down on ethanol costs. For other industries that use sugar, decreased sugar production costs will cut overall cost.

The second stage of the project (adding HTec 3) came to steady state, and as expected, xylose output was increased. FIGURE 1 shows the amount of glucose reached steady state and evened out. FIGURE 2 supports the xylose content also being raised.

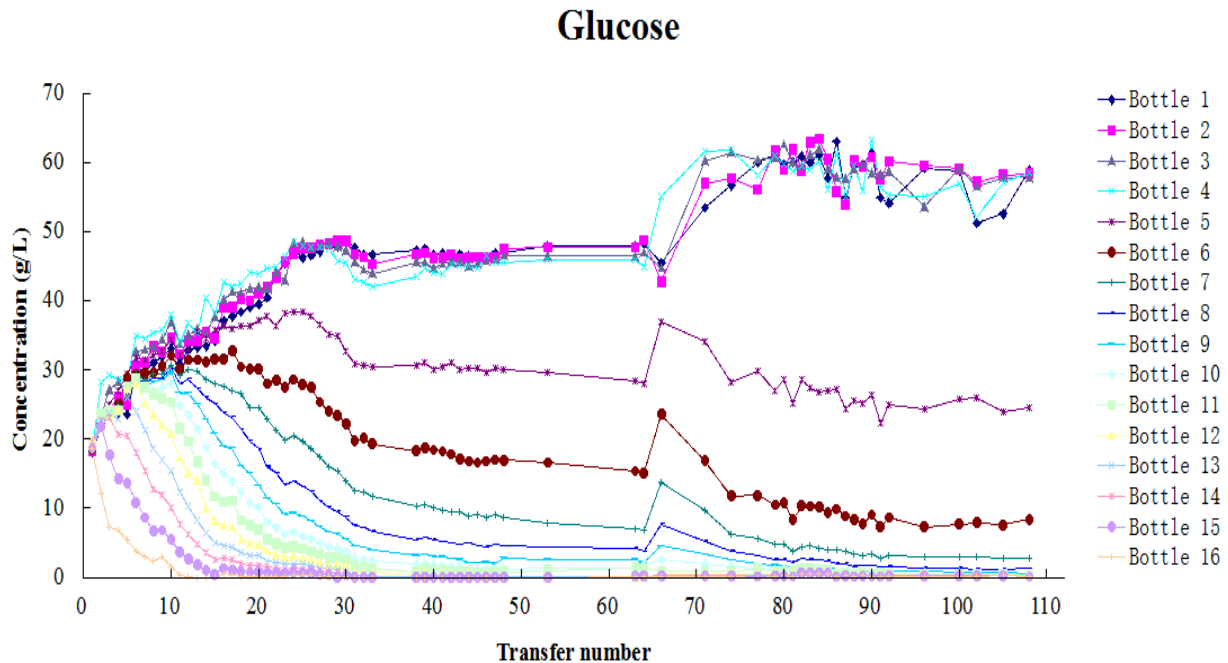


FIGURE 1: Glucose outputs throughout experiment.

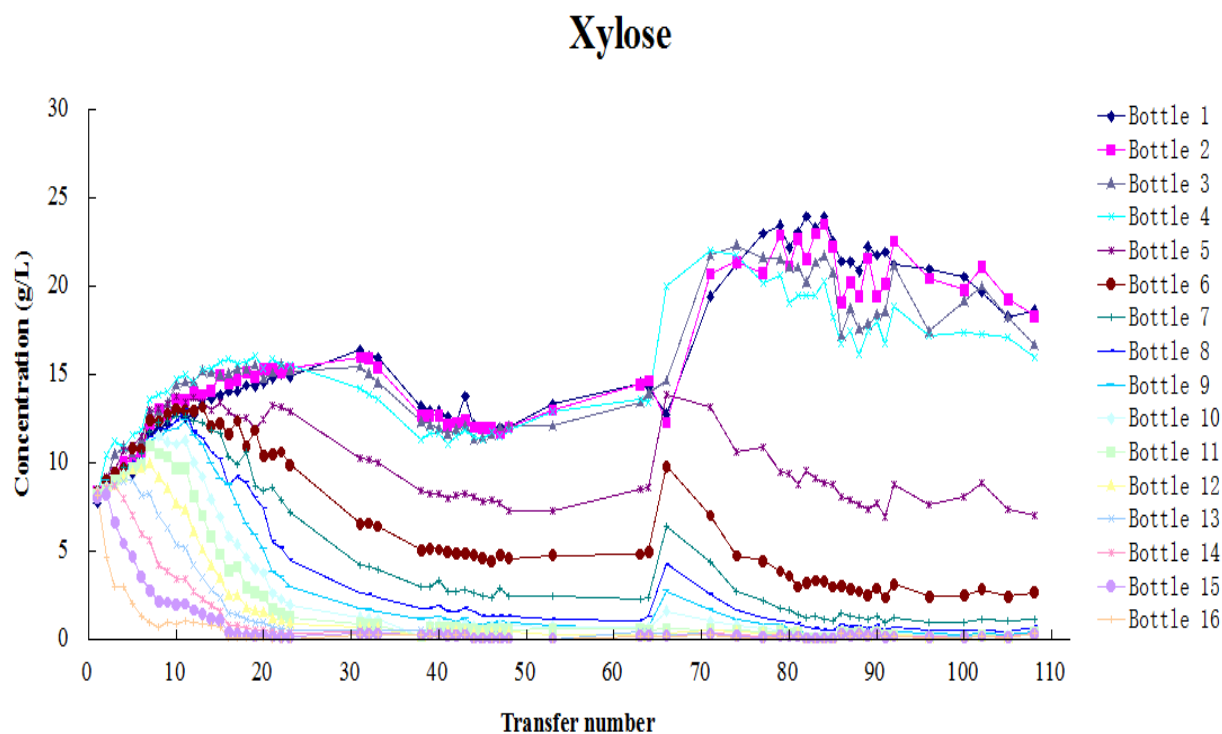


FIGURE 2: Xylose outputs throughout experiment.

FIGURE 3 and FIGURE 4 show the steady state concentration of glucose and xylose in Bottles 1-16 while adding an enzyme loading of 1mg CTec 3/g biomass. As the liquids are transferred from Bottle 16 towards Bottle 1, liquid sugar product moves down with it. As the pretreated biomass enters the system at Bottle 1 and moves down the system, it is digested into sugar. Therefore, a high concentration of sugar is present at Bottle 1, with concentrations decreasing from Bottle 1-16.

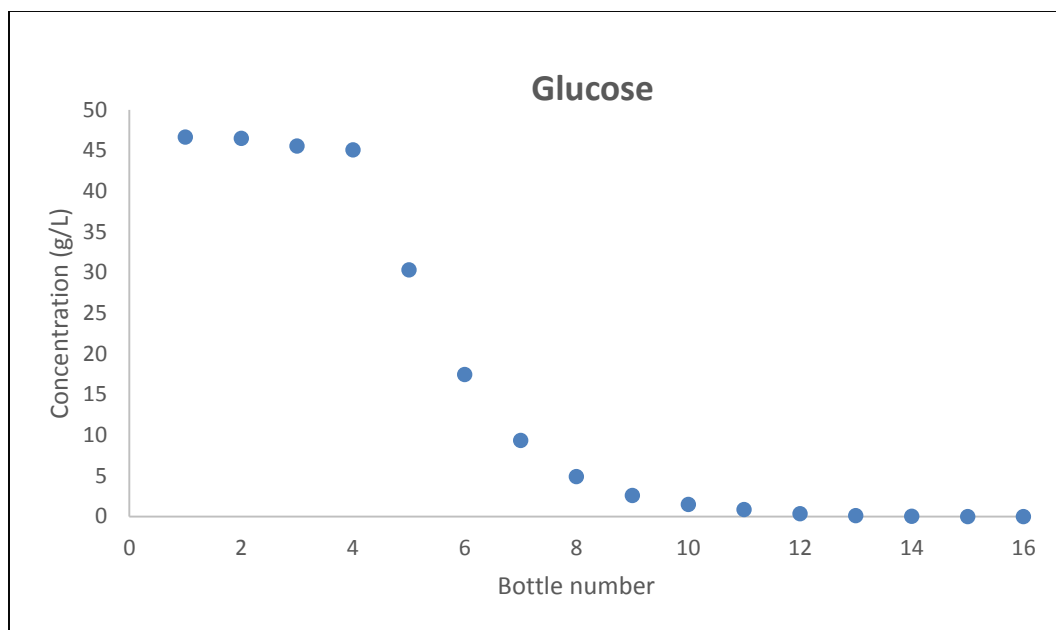


FIGURE 3: Glucose concentration in each bottle after adding CTec3.

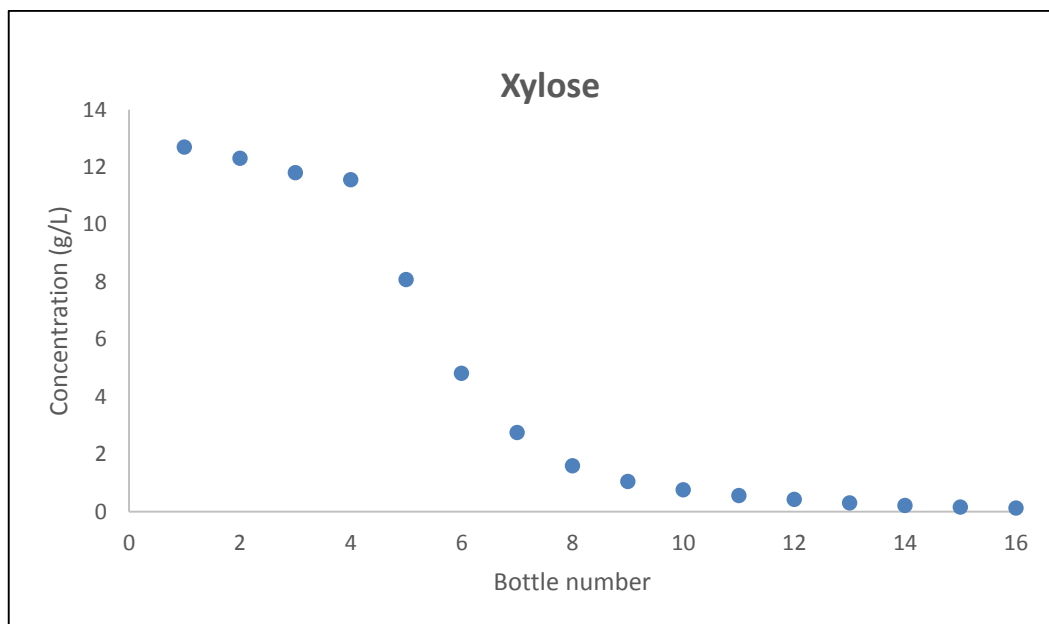


FIGURE 4: Xylose concentration in each bottle after adding CTec3.

FIGURE 5 shows the cumulative sugar output over time extrapolated from the mass of liquid product taken from Bottle 1 and the composition determined from HPLC analysis while using a

CTec 3 enzyme loading. An equivalent determination of biomass input was also calculated based on a fixed input of 10.941 g of biomass being added into Bottle 1 for each transfer that took place. TABLE 1 shows the yield of glucose and xylose obtained based on the curves plotted in FIGURE 5.

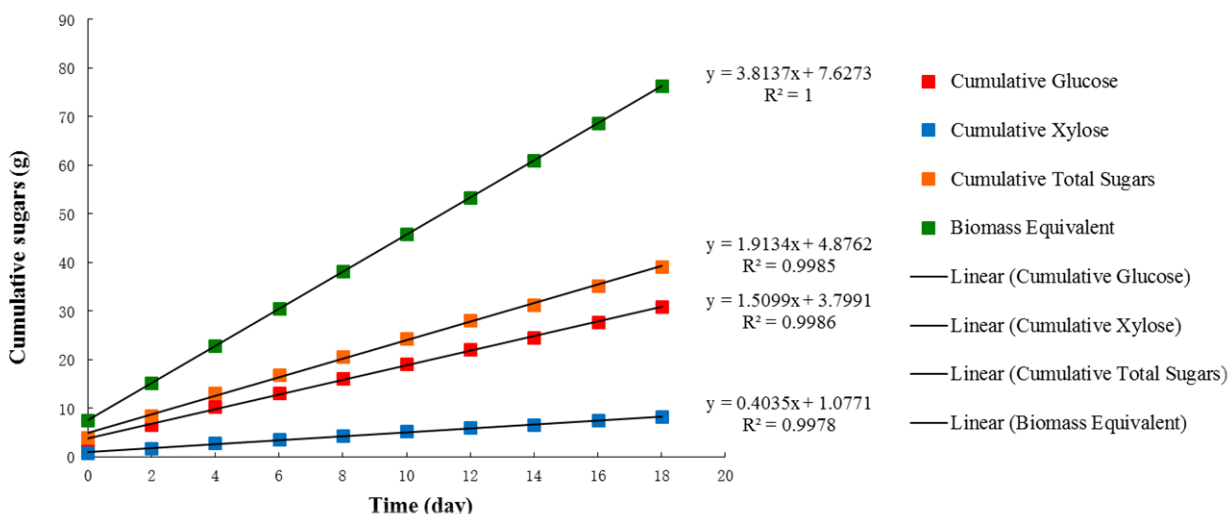


FIGURE 5: Sugar output with respect to days after adding CTec3.

	Yield (%)
Glucose	64
Xylose	30
Total sugar	51

TABLE 1: Sugar recovery after adding CTec3.

FIGURE 6 and FIGURE 7 show the steady state concentration of glucose and xylose in Bottles 1-16 while adding an enzyme loading of 1mg CTec 3/g biomass and 1mg HTec 3/g biomass. As the liquids are transferred from Bottle 16 towards Bottle 1, liquid sugar product moves down with it. As the pretreated biomass enters the system at Bottle 1 and moves down the system, it is digested into sugar. Therefore, a high concentration of sugar is present at Bottle 1, with concentrations decreasing from Bottle 1-16.

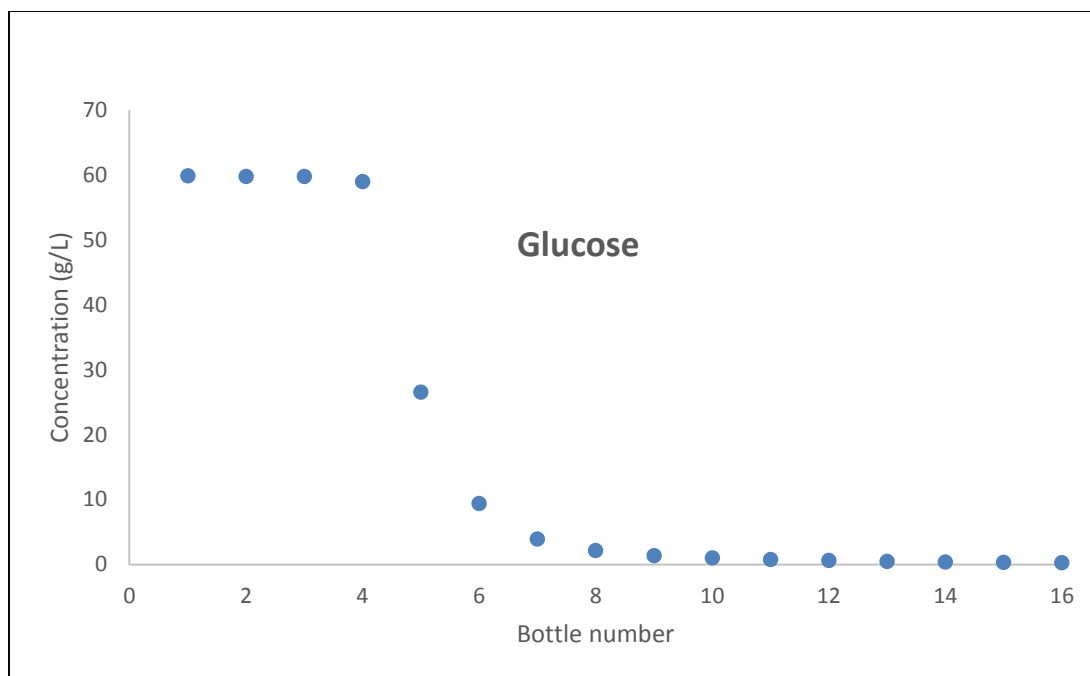


FIGURE 6: Glucose concentration in each bottle after adding CTec3 and HTec3.

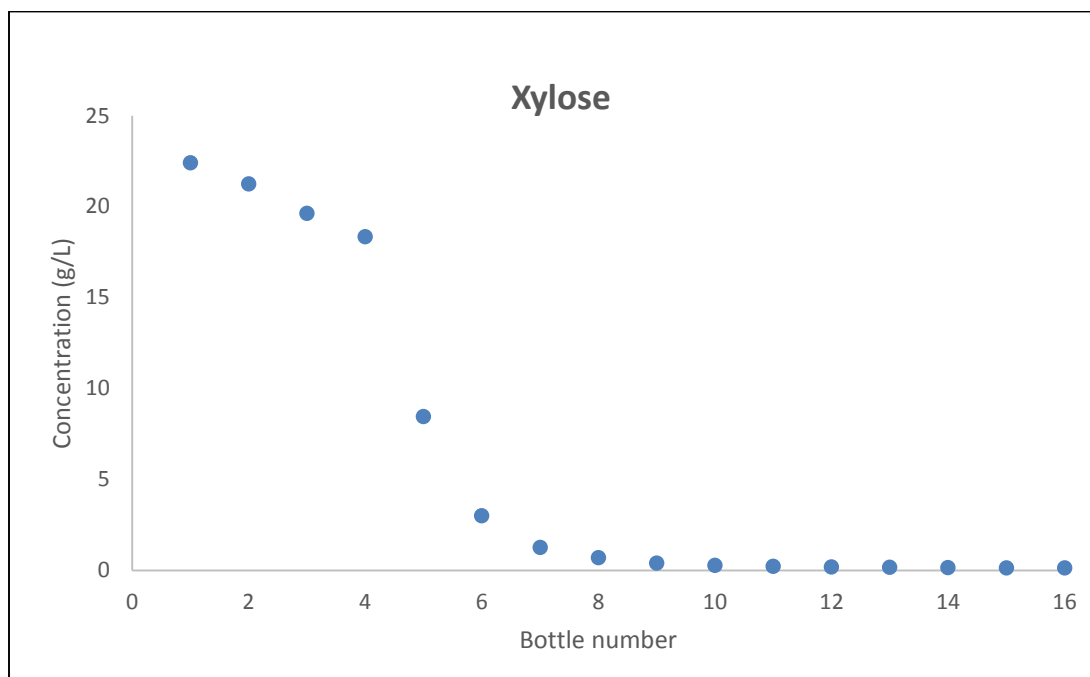


FIGURE 7: Xylose concentration in each bottle after adding CTec3 and HTec 3.

FIGURE 8 shows the cumulative sugar output over time extrapolated from the mass of liquid product taken from Bottle 1 and the composition determined from HPLC analysis while using both a CTec 3 and HTec 3 enzyme loading. An equivalent determination of biomass input was

also calculated based on a fixed input of 10.941 g of biomass being added into Bottle 1 for each transfer that took place. TABLE 2 shows the yield of glucose and xylose obtained based on the curves plotted in FIGURE 8.

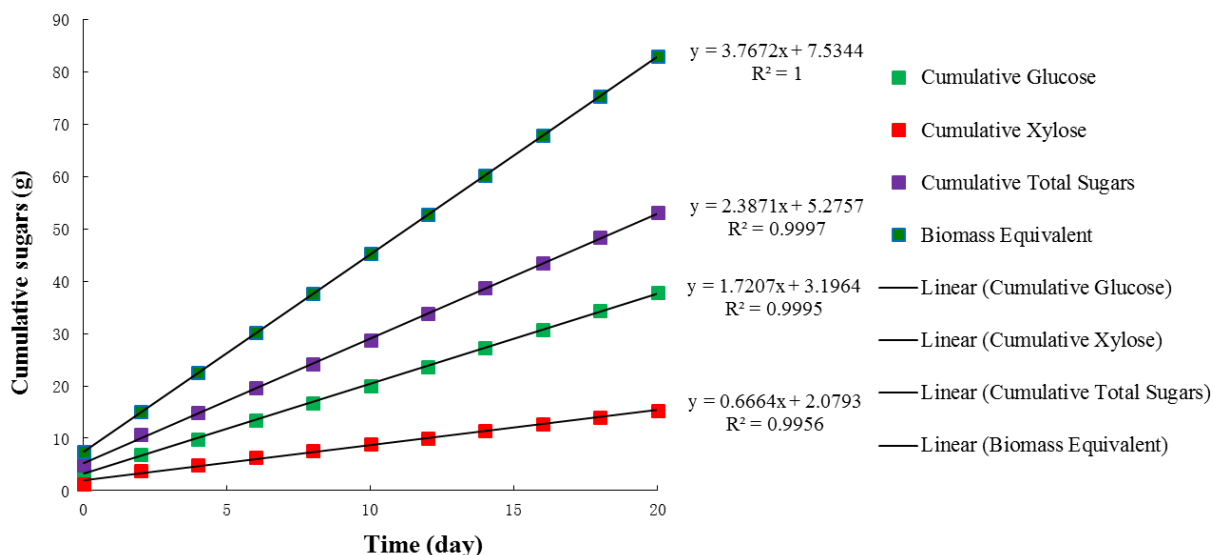


FIGURE 8: Sugar output with respect to days after adding CTec3 and HTec 3.

	Yield (%)
Glucose	72
Xylose	50
Total sugar	64

TABLE 2: Sugar recovery after adding CTec3 and HTec3.

FIGURE 9 shows how the countercurrent method's sugar concentration compares to that of an equivalent batch method setup. This is done for enzyme loadings of both CTec 3 alone and a combination of CTec 3 and HTec 3. In both cases, a 1 mg enzyme/g biomass loading is used. Both the batch and countercurrent setups used shock-pretreated biomass as well.

In the case of adding CTec 3 alone, both the countercurrent and batch method had comparable xylose concentrations. However, the countercurrent method had significantly higher glucose concentration (25%). In the case of adding both CTec 3 and HTec 3, the sugar concentrations came out closer. While the batch method had higher a xylose conversion (7%), the countercurrent method had a higher glucose concentration (7%).

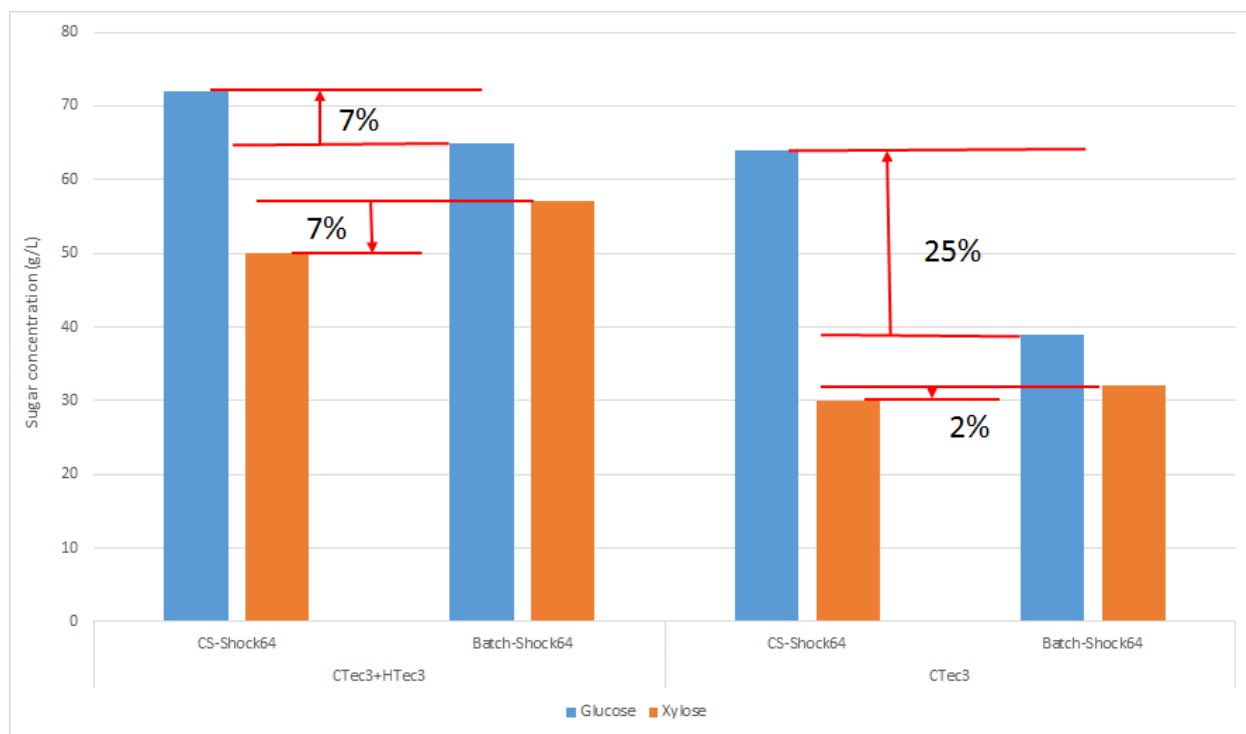


FIGURE 9: Comparison of countercurrent to batch method.

CHAPTER IV

CONCLUSIONS

Countercurrent saccharification of corn stover, using enzymes CTec 3 and a combination of both CTec 3 and HTec 3, was more effective in producing glucose than batch saccharification, but was marginally less effective in producing xylose. CTec 3 alone produced a competitive yield of glucose as well as a secondary yield of xylose. The addition of HTec 3 along with the original CTec 3 loading greatly increased the xylose yield along with a secondary increase in glucose yield. Both of these methods produced a higher overall sugar conversion than the batch method with the same enzyme-biomass ratio. The utilization of countercurrent saccharification can benefit the food industry, the chemical industry, and ethanol production. Glucose and xylose are fundamental sugars and are used in a variety of applications. If industries replaced the current batch method with a countercurrent method, they could produce more sugar using the same enzyme loading. The overall cost of production would then decrease and the price of sugar would eventually decrease as well. This price decrease of sugar would subsequently lower food, chemical, and ethanol consumer prices. A significant enough price decrease of ethanol in particular could make ethanol more competitive than gasoline, thereby replacing it.

REFERENCES

US National Library of Medicine National Institutes of Health. PubMed “Adsorptive control of water in esterification with immobilized enzymes. Continuous operation in a periodic counter-current reactor.” Department of Chemical Engineering, University of Virginia, 1999. Web. Sep. 2014

Oregon.gov. Biomass Energy: Cost of Production. Web. Jan. 2015.

Zentay, Agustin, Mark Holtzapple. “Countercurrent Enzymatic Saccharification of Cellulosic Biomass.” Print. 2014.