OPTIMIZATION OF SUGAR CONSUMPTION IN THE FERMENTATION OF TEMULOSE FOR ETHANOL PRODUCTION

A Senior Honors Thesis

by

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ABSTRACT

Optimization of Sugar Consumption in the Fermentation of Temulose for the Production of Ethanol

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Temulose is a wastewater stream created in the production of medium-density fiberboard. It has a high sugar content, and therefore cannot be released into standard wastewater systems. Current methods for disposal of the wastewater stream involve concentrating it in an energy-intensive process and selling it as a cattle feed supplement, but with energy prices rising there is an incentive to find higher-value uses. The purpose of this study is to examine the feasibility of using Temulose as a substrate for industrial ethanol production, using sugar consumption rates to determine the success of a fermentation. Three organisms were studied: *Zymomonas mobilis,* NRRL B-806; *Candida shehatae*, NRRL Y-12858; and *Saccharomyces cerevisiae*.

Shake-flask fermentations for *Z. mobilis* and *C. shehatae* were performed in triplicate for unamended, pH adjusted, and yeast extract amended Temulose at a sugar concentration of 20%. Fermentations with unamended Temulose showed little or no sugar consumption whatsoever, although the high sugar concentration of the Temulose may have affected the performance of the organisms. Fermentations with pH adjustment from 4.5 to 5.5 showed higher sugar consumption rates than yeast extract amended fermentations (1.16 mg/ml compared to 0.390 mg/ml for the first two dayof fermentation). Additionally, *Z. mobilis* was shown to have higher rates of sugar consumption for both amended fermentations (1.02 mg/ml compared to 0.75 mg/ml for the first day).

Fermentations using *S. cerevisiae* were performed with varying loading rates for yeast (0.5%, 1.0%, and 1.5%, w/v). Sugar consumption could not be determined, but ethanol concentrations up to 4% were observed after the first day of fermentation.

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TABLE OF CONTENTS

LIST OF FIGURES

I. INTRODUCTION[1]

As fossil fuel prices increase and environmental concerns gain prominence, the development of alternative fuels from agricultural wastes and industrial byproducts has become more important. The process of ethanol fermentation for the production of fuel hit its first major boom in the 1970's because of the gasoline shortage, but interest declined in the late 80's. More recently however, the combination of dwindling oil supplies, environmental awareness, and concern about America's dependence on foreign oil supplies has brought biofuels to the foreground again. Currently, the vast majority of America's ethanol is produced from corn, but there is a limit to the amount of ethanol that can be produced from this valuable cereal crop (Alterthum and Ingram, 1989). Subsequently, many researchers are searching for other fermentable materials that can replace or supplement corn in the production of ethanol. Cellulosic ethanol, created from high biomass crops such as switchgrass, is one of the most widely publicized sources, but another branch of possible raw materials includes sugary crops such as sugarcane or fruit.

A third possible substrate for ethanol production is Temulose®, a hemicellulose byproduct of the lumber industry also known as wood molasses. Temulose is produced at a Temple Inland medium density fiberboard facility in Diboll, Texas. During the production process, wood chips are boiled at high pressure and temperature, and soluble wood sugars are released when the pressure is quickly lowered. Figure 1 diagrams the process (D. Rogowski, personal communication, 25 July 2006).

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[1] This thesis follows the style and format of the *Transactions of the ASABE* (American Society of Agricultural and Biological Engineers).

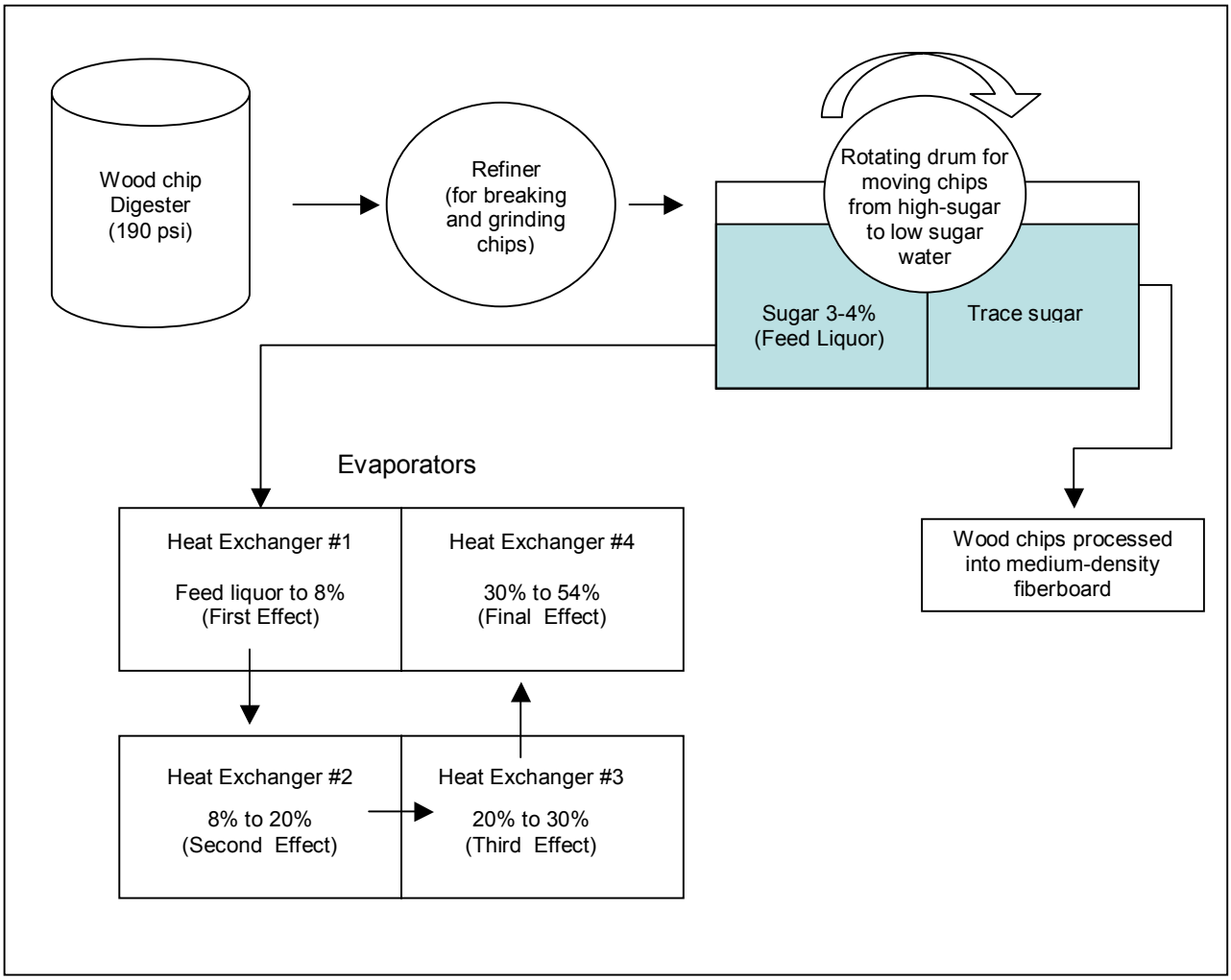

Figure 1. Current Production Process for Temulose

This process produces a stream of wastewater that has a sugar content of 4%--too high to be safely discharged into local streams. Currently, the water is concentrated to 54% Brix. The resulting thick, sticky liquid—Temulose—is sold as a dietary supplement for livestock. However, the condensation process is energy intensive, and Temulose's value as a supplement is barely enough to cover the energy costs.

Temple Inland is looking into alternate uses for their high-sugar waste stream. Because analysis has shown that the stream contains large percentages of mannose and xylose, one possible use is the production of specific sugar alcohols such as mannitol and xylitol, which have several important uses in medicine and as artificial sweeteners. This

process would only utilize a portion of the available sugars, however. Therefore, ethanol production is also being considered. In addition to the profit from selling ethanol, the energy inputs into the fermentation process would be close to zero, because the ethanol itself can be used for energy.

This project will not only help to find an economic solution to Temple Inland's waste water disposal problem, but it will provide a valuable fuel, open the door to ethanol production in similar industrial processes and add to the general pool of knowledge and experience in the growing field of renewable energy.

The primary goal of this study is to determine whether it is possible to produce ethanol from Temulose. The two secondary objectives are to identify what pretreatment, if any, is necessary in order to maximize ethanol production and to determine which of the selected microorganisms is more successful in producing ethanol and consuming sugar.

II. LITERATURE REVIEW

Raw Materials

M. Roehr (2001) describes many of the sugar and starch crops that would be suitable for industrial fermentation, including sugarcane, sugar beets, fruit, sweet potato, sweet sorghum, and even Jerusalem artichokes. The main problem with most of these materials is that most of them are either competing food crops or do not produce yields high enough for wide scale production. Waste products are another possible source of non-cellulosic ethanol that would not present competition for food crops. The industrial and food processing wastes described by Roehr include whey from the dairy industry and food industry wastes from the same crops discussed earlier. Whey may have possibilities as a fermentation supplement because of its high protein content, but with a sugar content of <5%, it is an unlikely raw material for ethanol production. Food industry wastes, which are often high in sugars or starches, are a much more promising area, but purifying and homogenizing the materials may present a problem. Instead of attempting to use their wastes for ethanol production, several food processing plants are employing anaerobic digestion for the production of methane.

Although the fermentable sugars in food wastes are easily accessible, their availability is limited to food production levels and the amount of waste that can be salvaged. For these reasons, cellulosic biomass has also become a raw material of interest in the search for sustainable fuels. Cellulosic biomass can be obtained from many sources, either in the form of waste from agricultural and forestry applications or from a high-biomass crop grown expressly for conversion to fuel. The three major components of cellulosic biomass are cellulose, hemicellulose and lignin. Cellulose is a long-chain, highly uniform polymer of glucose monomers joined by β-linkages. Hemicellulose is

another polysaccharide, although it is composed of many different sugar monomers, including xylose and mannose. Unlike cellulose, hemicellulose is not as uniform and tends to be more reactive. Both cellulose and hemicellulose can be fermented once their sugar monomers have been released through a process known as saccarification. Lignin, on the other hand, is a polymer of phenylpropylene subunits, and cannot be fermented. Lignin forms a crystalline protective structure around cellulose and hemicellulose in the structure of the biomass, is very difficult to degrade biologically, and interferes with the saccarification and fermentation of cellulose and hemicellulose. (Lynd, 1996) Because of this, several methods to chemically or physically degrade lignin are being investigated, including acid hydrolysis, alkaline hydrolysis, and steam explosion. In acid hydrolysis and alkaline hydrolysis, the pH of the biomass is raised or lowered to a level extreme enough to dissolve the crystalline structure of the lignin. The steam explosion, or autohydrolysis, method breaks up lignin and hemicellulose by "cooking" the biomass at high temperatures and pressures (160–260 °C and 0.69–4.83 MPa), then quickly lowering the pressure so that explosive decompression occurs. (Sun and Cheng, 2002) These and other pretreatment methods are a significant expense in the production of cellulosic ethanol.

Temulose fits into a sub-category of cellulosic ethanol sources known as waste sulfite liquors. The paper and lumber industries use many different processes to pre-treat wood, including acid and alkaline hydrolysis. Temulose, however, differs from many other waste sulfite liquors because its production does not involve the application of any strong chemicals that may inhibit fermentation. For example, spent sulfite liquor (SSL), a by-product of the sulfite pulping process, is a lignocellulosic hydrolysate that contains

both hexose and pentose sugars. It is produced in a manner similar to the fiberboard production process that creates Temulose, but the sulfite pulping process uses a strong acid solution instead of the steam explosion employed in the fiberboard process. (Roehr, 2001) Therefore, the two substances have similar sugar contents, but the differences in their production may influence the ability of fermentation organisms to grow and ferment. The advantage of waste sulfite liquors is that the paper production process already includes expensive Pretreatments, so taking advantage of the released sugars is a natural next step.

Fermentation Microbes

The microorganisms used to carry out the fermentation process are just as important as the substrate, and they have also been the target of much research. *Saccharomyces cerevisiae*, also known as brewer's yeast, is the most widely used fermentation microbe because of the baking and beer brewing industries, but its ability to produce ethanol for fuel is easily surpassed by many other organisms. (Roehr, 2001)

One of the most important fermentation microbes in the push for fuel ethanol is not a yeast at all, but the bacteria *Zymomonas mobilis*. When compared to yeasts, *Z. mobilis* not only offers higher sugar uptake and ethanol production rates, but it produces less biomass and has a higher ethanol tolerance. Unfortunately, *Z mobilis* also has several distinct disadvantages, including the inability to process the pentoses found in wood sugars or large polymers like starch and cellulose. Because bacteria are more genetically malleable than yeasts, however, several strains of *Z mobilis* have successfully been modified to express hydrolytic enzymes from other bacteria that enable it to utilize

glucose polymers. (Gunasekaran and Raj, 1999) More recently, researchers at the National Renewable Energy Lab in Golden, Colorado have produced strains with the ability to metabolize both hexose (six-carbon) and pentose (five-carbon) sugars. (Altintas et al., 2006) These important genetic modifications combine with *Z. mobilis'* native advantages to make it a very powerful fermentation microbe with great possibilities in many areas of biomass conversion.

Toivola et. al. performed a study in 1984 that screened 200 different species of yeast for their ability to ferment a combination of glucose and xylose. Interestingly enough, most of the yeasts that proved to be successful at fermenting xylose were originally isolated from wood insects, decaying wood, or other wood sources, including several species from the genera *Candida* and *Pichia*. Since then, the two most successful species in the study, *Pichia stipitis* and *Candida shehatae*, have become the subject of much research in the area of wood-based fermentations. (Toivola et. al, 1984) Studies using spent sulfite liquor have shown that both *C. shehatae* and *P. stipitis* are capable of fermenting SSL to produce greater ethanol yields than fermentations using only *S. cerevisiae*. (Bjorling and Lindman, 1988)

Fermentation Processes

The fermentation process itself can actually be even more important than the microorganisms used, and when industrial implementation is the end goal, the process must be considered. According to M. Roehr (2001) in *The Biotechnology of Ethanol,* four different fermentation operations are currently used in industry: batch, continuous, fed-batch, and semi-continuous. The batch process is the classical method that has stood the

test of time for hundreds of years, and is currently the most commonly used method of ethanol production. In batch processing, a cell slurry is grown separately from the fermentation substrate, and then slurry and substrate are combined in a reactor along with any required enzymes or nutrients. After the fermentation process is complete, the reactor is drained, sterilized, and refilled with a new batch. This method is popular not only because it requires low investment and labor costs, but because it is highly flexible and has a low risk of contamination or cell mutation due to the relatively short cultivation times involved. The disadvantages, however, make the batch process most suitable for smaller scale operations.

The fed-batch process combines the batch and continuous methods, and is most suitable for larger operations where mutation of the fermentation microbe is a risk or the substrate has an inhibitory effect on the process. The process begins in the same way as a batch fermentation, but over the course of the fermentation a feed solution containing cell culture, nutrients, and substrate is added at controlled intervals. The feed solution is added so that incoming substrate matches substrate consumption in the fermentation, allowing the concentration to remain low enough to prevent inhibition. The process continues until the ethanol concentration reaches inhibiting levels, at which point the tank is drained and the process is repeated. This process provides high ethanol yield because environmental conditions can be controlled and cultivation time is well-defined.

The semi-continuous process is another compromise between the batch and continuous methods, although it falls closer to continuous. As in fed-batch processes, a feeding solution is slowly added to the fermentation, although it is not tightly controlled with the goal of keeping the substrate low. Instead, as the volume of the fermentation

increases, it overflows from the first reactor into a second. Then the first reactor is left to ferment, and the second reactor is gradually filled with substrate, nutrients and cell culture. This process continues through several more reactors, and meanwhile the first reactor can be harvested and cleaned without losing any productivity due to inactive reactors or microbial lag time. Unfortunately, there is still a high investment cost because of the larger reactor volumes, and the risk of contamination is fairly high. (Roehr, 2001)

Continuous fermentation virtually eliminates the time required for constant cleaning and lag phase. The fermentation solution, containing substrate, nutrients and cell medium are continuously added to an agitated reactor with active microbes. The substrate is consumed, and a product solution of water, ethanol, cells and residual sugar is taken from the top of the reactor. This method is most effective for large-scale operations, and it requires the least manpower because it is almost completely automated. There is also less wear on the instruments from constant sterilization and a reduced risk to personnel because of limited exposure. The continuous process does require a uniform substrate composition, because the automation compromises flexibility. Also, the risk of mutation from outside contamination or internal adaptations is very high due to the long cultivation times, and constant sterilization of the media can be expensive. (Roehr, 2001)

III. METHODS AND MATERIALS

Substrate

The Temulose used in this study was obtained from the Temple Inland plant in Diboll, Texas. After collection from the plant, the samples were frozen to prevent contamination and stored for approximately one month. In the fermentations using *Z. mobilis* and *C. shehatae,* second effect Temulose was used (18% Brix). In the fermentations using *S. cerevisiae,* first effect Temulose was used (9.5% Brix).

Microorganisms and Culture Conditions

The three microorganisms used in this study were *Zymomonas mobilis*, *Candida shehatae,* and *Saccharomyces cerevisiae*. The *Saccharomyces cerevisiae* used in the fermentations was commercially available, taken from a home brewing kit in active dry form. *Zymomonas mobilis*, NRRL B-806 was obtained from the ARS Culture Collection and maintained on solid media containing 20 g/L glucose, 5 g/l yeast extract, and 20 g/L agar. *Candida shehatae* var. *shehatae*, NRRL Y-12858 was also obtained from the ARS Culture Collection, and maintained on solid media containing 20 g/L glucose, 20 g/L peptone, 10 g/L yeast extract, and 20 g/L agar. Solid cultures were maintained at 4°C.

Inocula Preparation

Inocula were prepared for *Z. mobilis* and *C. shehatae* by transferring a loopfull of cells from the agar plate to 10 mL of inoculum media. The inoculum media for both organisms had the same nutrient composition as the solid media: 20 g/L glucose and

5 g/L yeast extract for *Z. mobilis*, and 20 g/L glucose, 20 g/L peptone and 10 g/L yeast extract for *C. shehatae.* Inocula were kept in a loosely capped test tube, incubated for 48 hours at 35°C, and vortexed before use.

Saccharomyces cerevisiae was not prepared in a liquid incocculum, but added directly to the fermentations in dry form.

Fermentation

Fermentations for *Z. mobilis* and *C. shehatae* were carried out in 250 mL Erlenmeyer flasks, using 125 mL of Temulose. For each organism, a set of nine fermentation flasks was used. Three of the flasks contained Temulose that had been amended with 3.75 g/L yeast extract, in order to determine whether non-carbohydrate nutrients were a limiting factor in microbial growth. Three flasks were amended from a pH of 4.5 to a pH of 5.5 using NaOH in order to determine if low pH was a limiting factor, and the last three flasks contained unamended substrate as a control.

After the substrate was prepared, 2 mL of inoculum was added to each flask, and the mouth of the flasks were tightly covered with aluminum foil, which would restrict but not eliminate gas exchange. The flasks were incubated at 31.6°C in a Model G25 incubator-shaker from New Brunswick Scientific. The fermentations continued for 7 days, samples were taken approximately every 24 hours, and frozen immediately after extraction.

Fermentations for *S. cerevisiae* were performed in sealed 2L PET bottles. Each bottle contained 500 ml of Temulose inoculated with dry yeast at concentrations of 0.5% (w/v), 1.0% (w/v), 1.5% (w/v), and 0% in triplicate. Bottles were incubated at 31.6°C in a

Model G25 incubator-shaker, samples were taken every 24 hours for 5 days, and frozen after extraction.

Analytical Methods

Sugar Analysis

Thawed fermentation samples were filtered through a 0.2 µm filter into HPLC autosampler vials. Concentration of sugars, including glucose, xylose, mannose, arabinose, cellobiose, and galactose, were measured by HPLC using a ConstaMetric 3200 solvent delivery system from LDC Analytical, Shodex SP 0810 column and RI 2000 refractive index detector from Lab Alliance. The column was operated at 75°C and the mobile phase was water. Unfortunately, there were several problems with this HPLC setup. An unidentified substance at high concentration eluted shortly before the sugars of interest, and the tail of its peak interfered with the peaks for glucose, maltose, xylose and galactose. Mannose was the only sugar which peaked at a time late enough to escape the effect. Figures 2 and 3 show sample chromatographs of the sugar standards compared to the actual fermentation samples.

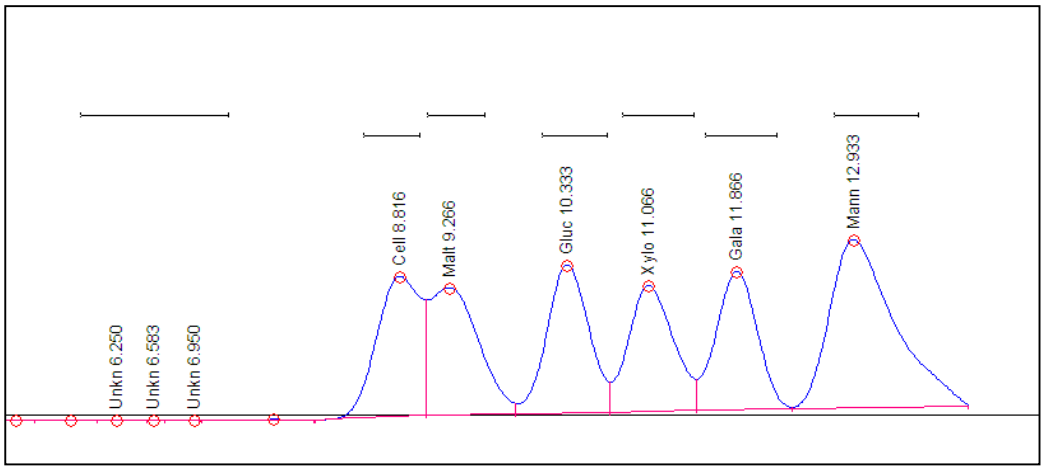

Figure 2. Sugar Standard Chromatograph, all sugars at 5 mg/ml

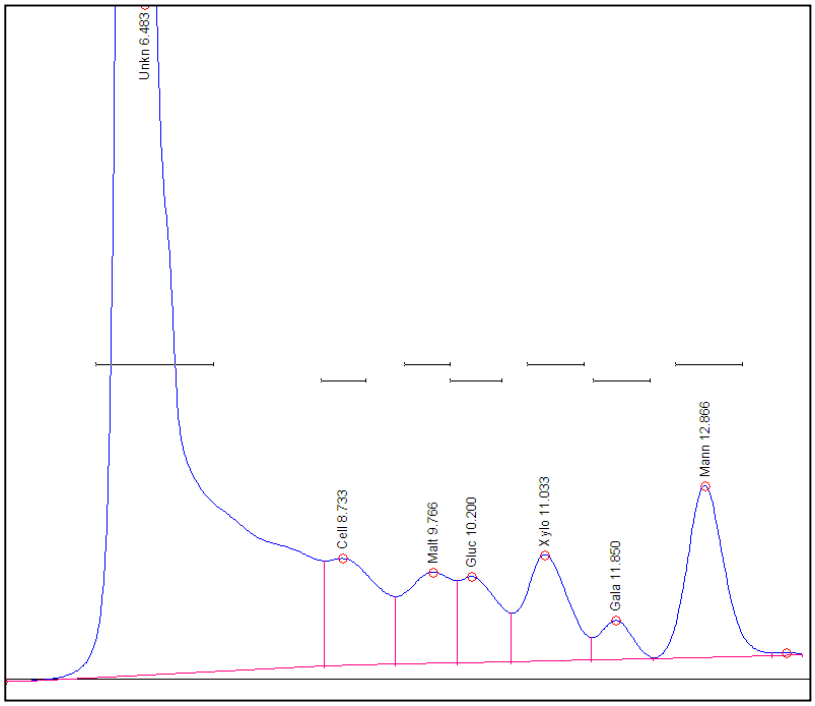

Figure 3. Fermentation Sample Chromatograph, from pH amended *Z. mobilis* fermentation, Day 5

Two other factors must be taken into effect when examining the data for mannose concentration. When examining the standard peaks, it was determined that mannose and arabinose eluted at the same time, resulting in a single peak with twice the area of the other standards. However, arabinose has a low concentration in Temulose compared to mannose, so it should not have a significant effect on the results. When converting from chromatograph area to actual concentration, this will be accounted for by halving the slope of the standard curve. The other factor is that ethanol was determined to elute shortly after mannose and arabinose, so increasing ethanol levels may create conflicting results, and in some cases only the early consumption data is usable.

Ethanol Analysis

In order to determine the level of ethanol in fermentation samples, headspace analysis using gas chromatography was used. 10 ml of thawed sample were sealed in a 20 ml vial and placed in a water bath at 70°C. After the temperature equalized, 10 µl of the sample headspace was injected. The column used was a MXT-1, (100% Dimethyl polysiloxane, non polar phase, Crossbond) and the gas chromatograph was a model 8610C from SRI Instruments.

Ethanol analysis for *Z. mobilis* and *C. shehatae* fermentations did not show significant levels of ethanol, probably due ethanol's volatility and the length of time that the samples were stored before analysis.

IV. RESULTS

Sugar Composition

A previous analysis by Covance Laboratories in 1998 determined that the percentage of mannose in Temulose was much higher than other sugars. Figure 4 gives a comparison between the sugar composition from this study and the previous composition data (Covance Laboratories, Inc. unpublished data, 1998).

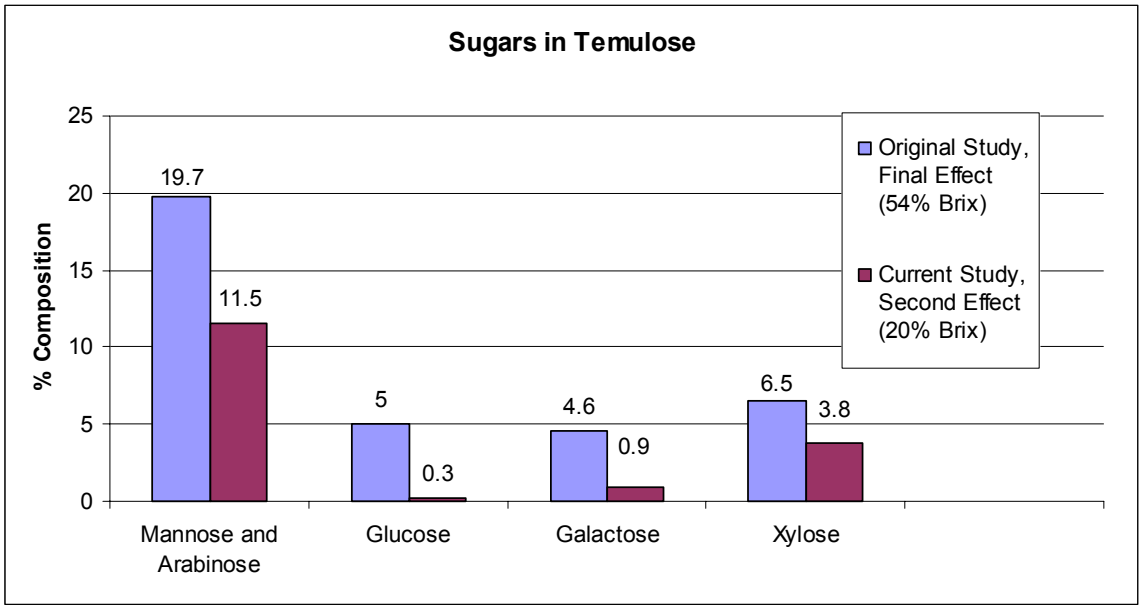

Figure 4. Sugar Profile of Temulose (Mannose and Arabinose combined for comparison).

Because mannose was the only sugar that could be analyzed without interference from tailing substances and because of its high concentration when compared with other sugars, it will be used as an indicator of sugar consumption in this study.

Pretreatment

Both yeast extract and pH amended fermentations showed improved sugar consumption when compared to the unamended fermentations, which showed little or no consumption whatsoever. Fermentations with pH amendment, however, showed higher

rates of consumption than fermentations with yeast extract amendment. Table 1 compares the pH and yeast extract amended fermentations.

	Amendment	
	pH (mg/ml-day)	YE (mg/ml-day)
Z. mobilis	1.337 (0.109)	0.4182 (0.0498)
C. shehatae	0.9837 (0.151)	0.03977 (0.0599)
Average	**1.160 (0.227)**	**0.2290 (0.213)**

Table 1. Mannose consumption for pH and YE amended fermentations (standard deviation in parentheses)

The data for pH amended fermentations is based on the second day of fermentation, because after that point the data becomes unusable. Data for yeast extract amended fermentations is taken from all seven days of the fermentation.

Average mannose consumption for the *Z. mobilis* yeast extract fermentations was 2.51 mg/ml over 7 days of fermentation, which gives a decrease of 18.9%. Figure 5 shows the daily sugar consumption for this fermentation. Although data for the pH fermentations is not usable past the first day of fermentation, average mannose consumption after the first day of fermentation is 1.34 mg/ml—which gives a decrease of 13.8%—more than half of the decrease for the entire seven days of the yeast extract fermentations.

Z. mobilis* and *C. shehatae

Sugar consumption data for *Z. mobilis* and *C. shehatae* is shown in Figure 6. *Z. mobilis* showed consistently higher sugar consumption than compared to *C. shehatae*, although the differences were greater between yeast extract amended fermentations than pH amended fermentations.

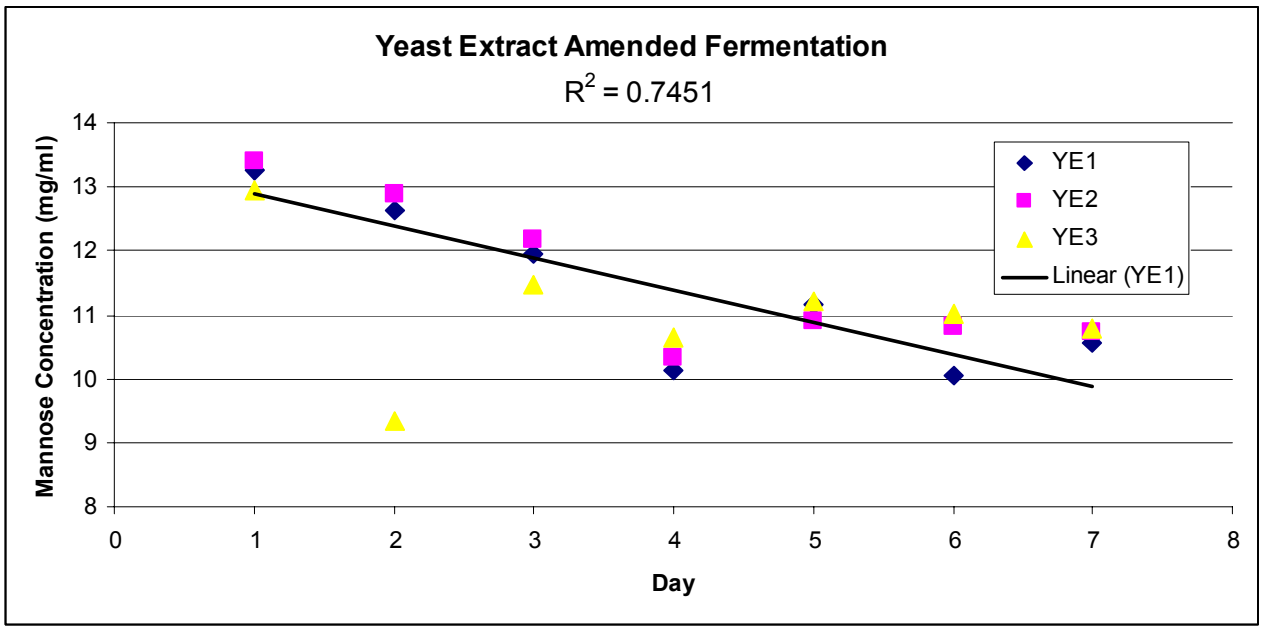

Figure 5. Daily Mannose Concentration for Yeast Extract Amended Fermentations

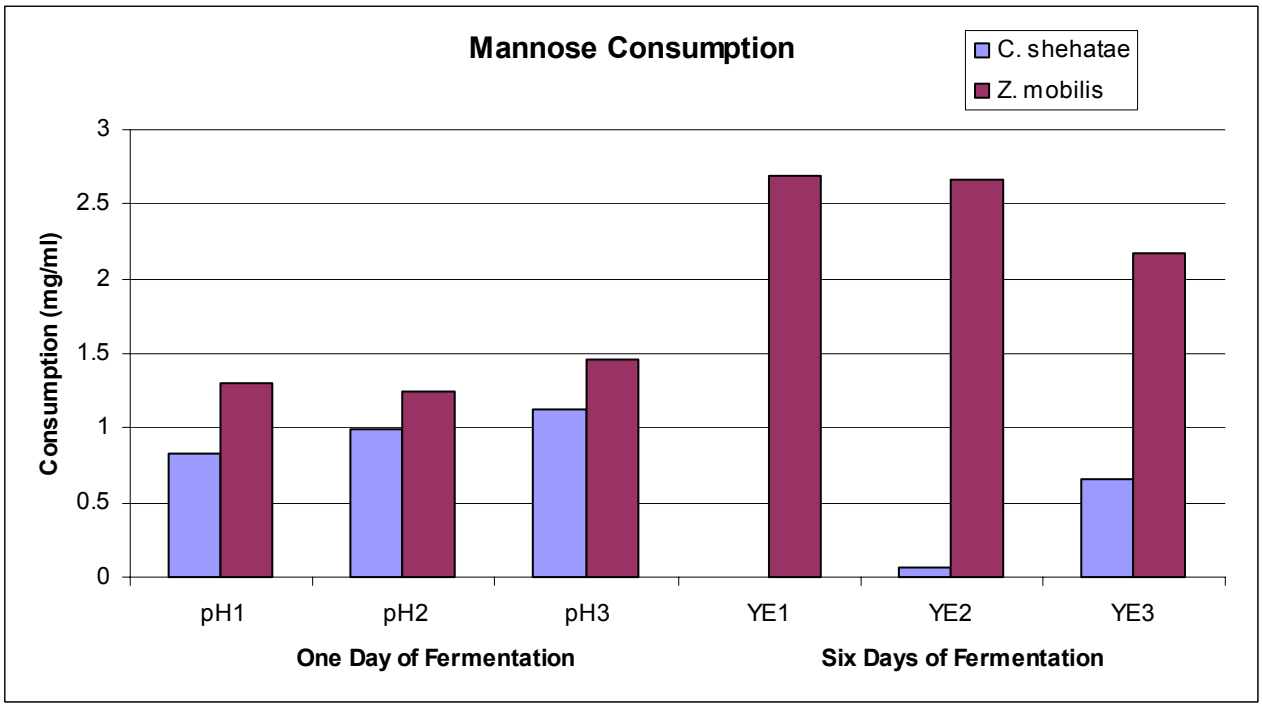

Figure 6. Observable Mannose Consumption.

Mannose was the only sugar that showed any significant decrease over the course of the fermentations. Figure 7 shows all sugars over the course of the yeast extract fermentation for *Z. mobilis*.

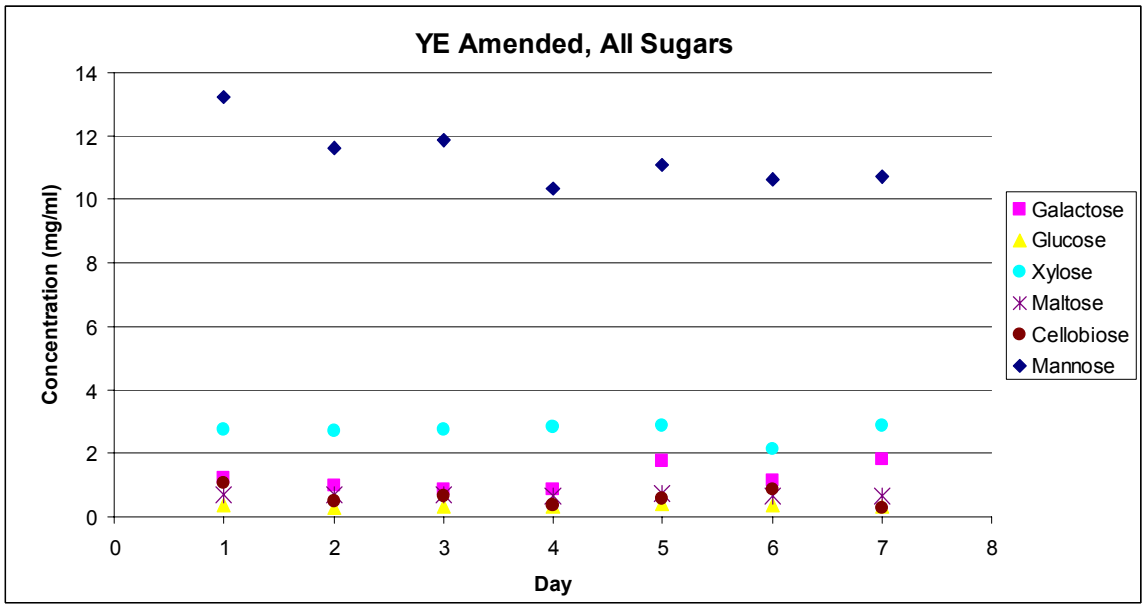

Figure 7. Concentration of Sugars Other than Mannose in *Z. mobilis* Yeast Extract Fermentation

No decreasing trend was apparent in the non-mannose sugars over the course of this or any other fermentation, and no linear correlation above $R^2 = 0.40$ was found.

Theoretical Ethanol Production

Although the ethanol production for *Z. mobilis* and *C. shehatae* fermentation could not be analyzed, it is possible to estimate the theoretical ethanol yield from sugar consumption. The equation for conversion of sugar to ethanol is given in Equation 1 (Johnson-Green, 2002).

$$\underset{\text{100 g Mannose}}{C_6H_{12}O_6} \rightarrow \underset{\text{51 g Ethanol}}{2C_2H_5OH} + \underset{\text{49 g Carbon Dioxide}}{2CO_2} \qquad \textbf{Eq. 1}$$

Using this information, the ethanol produced for *Z. mobilis* fermentations is given in Table 2 below, assuming a 40% conversion efficiency.

	Mannose Consumed (mg/ml)					
	pH1	**pH2**	**pH3**	**YE1**	**YE2**	**YE3**
Z. Mobilis	1.300	1.252	1.460	2.699	2.664	2.165
C. shehatae	0.829	0.993	1.129	0.000	0.064	0.652
Averages	1.064	1.122	1.295	1.349	1.364	1.408
Ethanol Produced (mg/ml)						
Z. Mobilis	0.265	0.255	0.298	0.551	0.544	0.442
C. shehatae	0.169	0.203	0.230	0.000	0.013	0.133
Averages	0.217	0.229	0.264	0.275	0.278	0.287

Table 2. Mannose Consumed and Theoretical Ethanol Production (Note: pH fermentation data is from one day of fermentation, yeast extract data is from six days of fermentation)

Fermentations with *S. cerevisiae*

In the fermentations using *S. cerevisiae,* no significant decreasing trend was observed in sugar consumption, although this was probably a result of difficulties with HPLC methodology instead of a lack of consumption, because gas chromatography analysis showed that ethanol was being produced. Figure 8 shows the levels of ethanol in

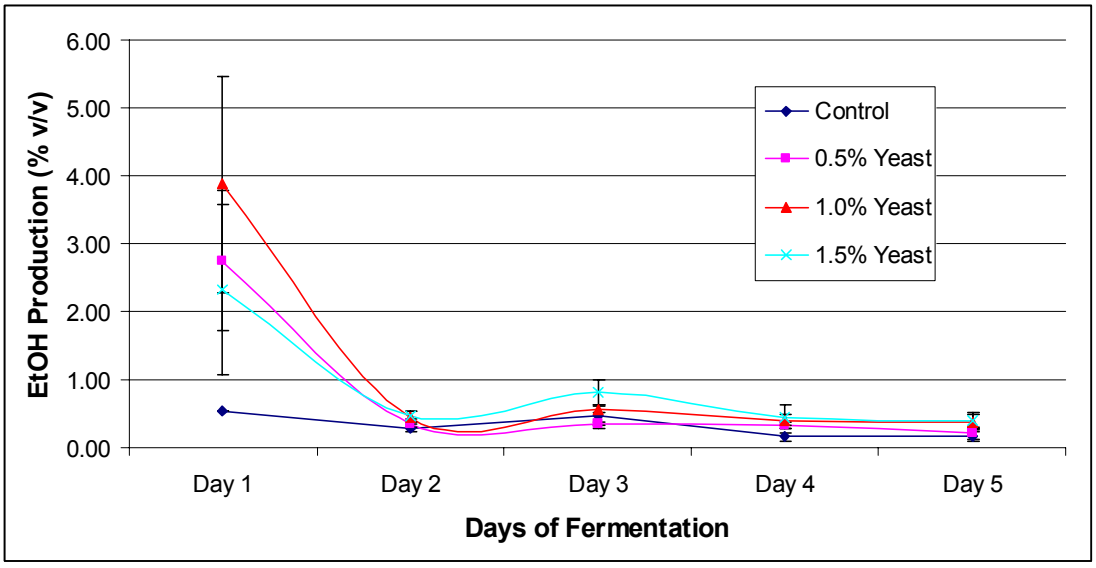

Figure 8. Ethanol Levels in *S. cerevisiae* Fermentation Samples (Error bars represent standard deviation)

fermentation samples for each day of the fermentation. The sharp decrease in ethanol levels after the first day of fermentation is probably a result of ethanol's volatility, but these results do show that fermentation was occurring.

V. CONCLUSIONS

Although actual ethanol production cannot be determined solely from sugar consumption data, it is an indication of the success of an organism in ethanol production. When comparing pretreatments, it was shown that although both yeast extract amendation and pH amendation improve sugar consumption rates above the levels of unamended Temulose, pH amendation provided sugar consumption rates up to five times higher than rates for yeast extract amendation.

When comparing sugar consumption for the two organisms used, mannose consumption for *Z. mobilis* was consistently higher than for *C. shehatae*. Theoretical ethanol production was calculated from the sugar consumption data and, assuming 40% conversion efficiency, yields as high as 0.55 mg/ml were estimated.

Although we were not able to trace sugar consumption for fermentations using *S. cerevisiae*, ethanol analysis showed significant ethanol levels in the first day of fermentation, up to 4%. This not only shows that was fermentation occurring in these trials, but that ethanol is even more volatile than we accounted for in our methodology. The decrease in ethanol concentrations over time may mean that our inability to detect the presence of ethanol in fermentations with *Z. mobilis* and *C. shehatae* was a result of the methodology, and further studies are needed to evaluate this substrate for ethanol production.

VI. RECOMMENDATIONS

Further research in this area is necessary before design recommendations can be made. Because it showed the highest sugar consumption rates, *Z. mobilis* fermentations with pH adjustment will probably produce the highest ethanol yield. Future studies should consider substrate concentration in addition to pH, because the 20% sugar Temulose used in this experiment was fairly high and probably inhibited cell growth and productivity. Temulose at lower sugar concentrations, as in the *S. cerevisiae* fermentations, may produce better results because the concentration of any inhibititory compounds will also be decreased. First effect Temulose (8-9% Brix) also requires less energy to produce, lowering the overall energy requirements for the fermentation process.

Another advantage of using *Z. mobilis* as a fermentation organism is that native strains only process six carbon sugars such as glucose, galactose and mannose. The five carbon sugar xylose could then be converted to xylitol, another high-value product.

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APPENDIX A

Microbial Cultures

YPD medium for yeast cultures:
20 g/L Peptone
10 g/L Yeast Extract
20 g/L Agar (for plates only)
Add H2O to 950 ml. Adjust to pH 6.5 if necessary, then autoclave. Allow medium to cool to ~ 55°C and then add glucose to 2% (50ml of a sterile 40% stock solution). Adjust the final volume to 1 L if necessary.

Taken from http://www.protocol-online.org/cgi-bin/prot/view_cache.cgi?ID=3932

Zm medium for *Z. mobilis* cultures:
5 g/L Yeast Extract
20 g/L Agar (for plates only)
Add H2O to 950 ml. Autoclave, then allow medium to cool to ~ 55°C and add glucose to 2% (50ml of a sterile 40% stock solution). Adjust the final volume to 1 L if necessary.

Adapted from Handbook of Microbiological Media, 2nd Edition, 1997 by Ronald M. Atlas, CRC Press, pg 1587.

All media, test tubes and other instruments were sterilized at 121°C for 15 minutes.

Most microbiological procedures were performed under a laminar flow hood. Plates and inocula were incubated at 35°C, then stored at 4°C for up to a month.

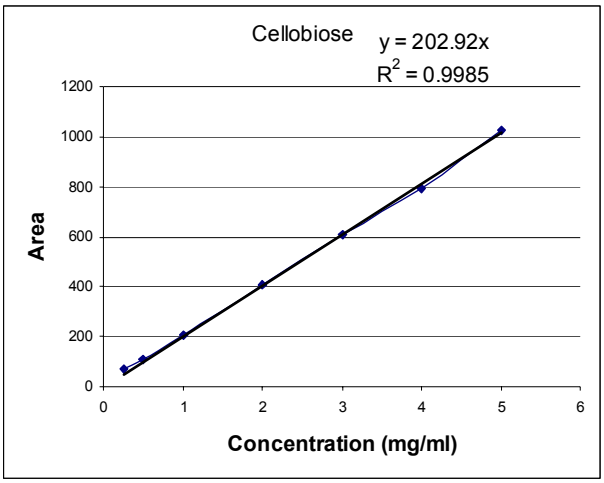
APPENDIX B

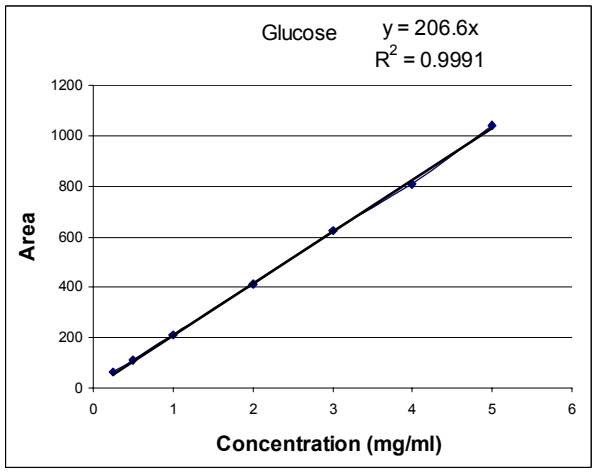
HPLC Data and Procedures

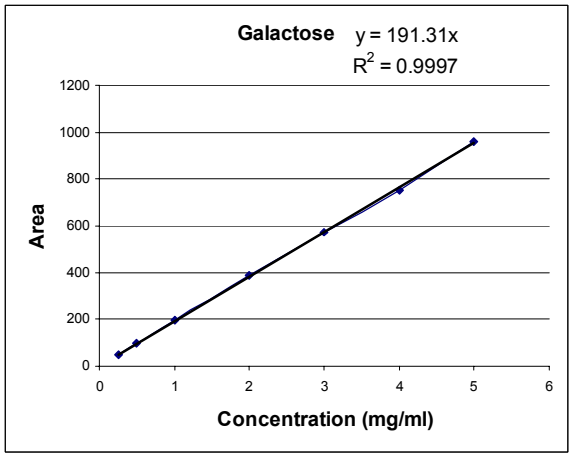
HPLC Settings:

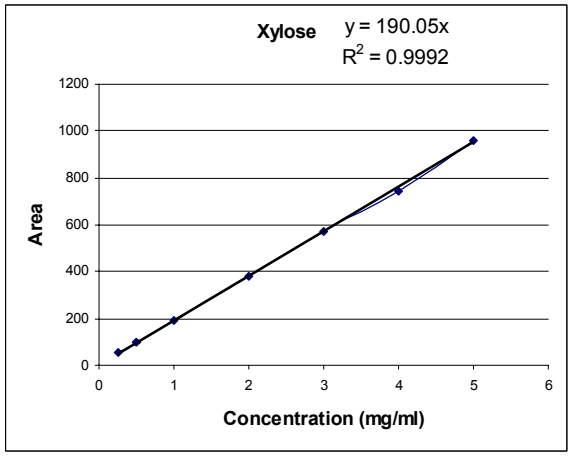
Column Temperature	75°C
Flowrate	8 ml/min
Mobile phase	Ultra-pure water, HPLC grade
Column	Shodex SP 810

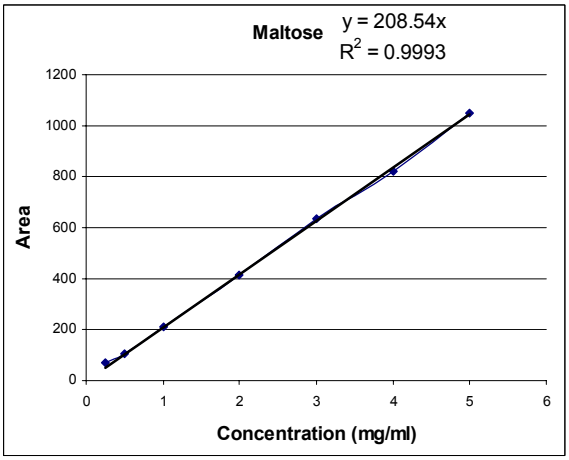
Standard Curves

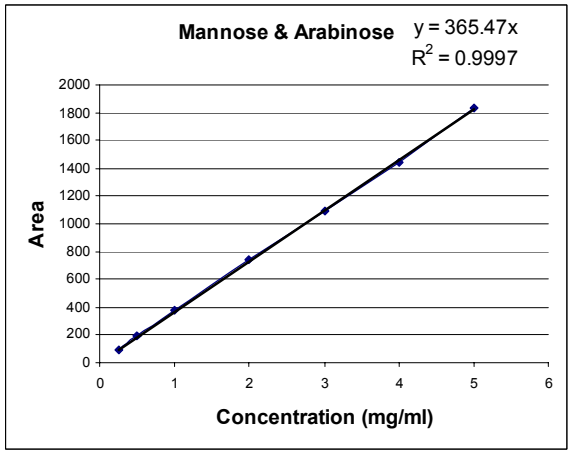












Concentration Data for Each Replication

	Unamended 1 Z. mobilis					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.0000	0.8488	0.2660	3.7801	0.9756	11.3896
Day 2	0.0000	0.7842	0.3077	2.3924	1.6825	10.4503
Day 3	0.3782	0.7536	0.2754	2.1539	0.8788	11.5102
Day 4	0.6909	0.8259	0.3093	2.4231	0.8863	11.2012
Day 5	0.4833	0.7698	0.3212	2.7018	2.2413	11.2491
Day 6	0.4365	0.8008	0.3289	2.5047	2.0821	11.7139
Day 7	0.4322	0.7396	0.3623	2.3996	1.0004	11.2725

	Unamended 2 Z. mobilis					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.0000	0.8488	0.2660	3.7801	0.9756	11.3896
Day 2	0.3085	0.6819	0.3558	2.1253	0.8603	10.1600
Day 3	0.5354	0.7464	0.4463	2.8125	1.0126	8.7021
Day 4	0.3056	0.6471	0.3373	2.5894	0.9018	11.4114
Day 5	0.4423	0.7249	0.3842	2.3392	1.9749	10.9195
Day 6	0.4839	0.7197	0.4005	2.0583	1.6908	11.2316
Day 7	0.4875	0.7226	0.3490	2.0607	1.0663	11.3214

	Unamended 3 Z. mobilis					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.0000	0.8488	0.2660	3.7801	0.9756	11.3896
Day 2	0.3797	0.7640	0.2952	2.4161	0.9107	11.0901
Day 3	0.6476	0.8528	0.2231	2.8982	1.0071	5.3418
Day 4	0.3587	0.7383	0.1998	2.6741	0.9299	11.2644
Day 5	0.4506	0.7797	0.3032	2.6198	2.1678	11.3159
Day 6	0.4005	0.7717	0.2897	2.3312	1.9186	11.6087
Day 7	0.3910	0.7678	0.3607	2.5255	1.0438	11.2927

	Unamended Summary, Z. mobilis					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.0000	0.8488	0.2660	3.7801	0.9756	11.3896
Day 2	0.3441	0.7229	0.3255	2.2707	0.8855	10.6250
Day 3	0.5915	0.7996	0.3347	2.8554	1.0099	7.0220
Day 4	0.3322	0.6927	0.2686	2.6317	0.9159	11.3379
Day 5	0.2976	0.7844	0.3178	2.9130	1.7061	11.2083
Day 6	0.2948	0.7586	0.3326	2.2606	1.7640	11.0969
Day 7	0.4189	0.7480	0.3284	2.2467	0.9963	11.3748

	pH Amended 1, Z. mobilis					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.5109	0.9778	0.3179	2.1161	0.9746	9.6801
Day 2	0.8084	0.6124	0.3302	2.0980	0.9596	8.3799
Day 3	0.4521	0.5819	0.3679	2.2498	1.6273	8.5294
Day 4	0.6433	1.0479	0.2111	2.3550	1.1327	9.3387
Day 5	0.4776	0.4873	0.4093	3.1219	0.8884	9.2935
Day 6	0.4195	0.5860	0.5204	2.7787	0.8358	9.5575
Day 7	0.4433	0.5864	0.5300	2.8850	0.9059	9.9541

	pH Amended 2, Z. mobilis					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.5109	0.9778	0.3179	2.1161	0.9746	9.6801
Day 2	0.0000	0.5776	0.3261	2.0256	1.5245	8.4284
Day 3	0.8225	0.5802	0.3251	2.1756	0.9714	8.5919
Day 4	0.0000	0.9940	0.6591	0.4369	2.3352	0.0000
Day 5	0.4353	0.4178	0.0000	2.8802	0.8878	9.0232
Day 6	0.4676	0.5356	0.4811	2.9575	0.8881	9.5672
Day 7	0.4239	0.5052	0.4327	2.9907	0.9712	8.9944

	pH Amended 3, Z. mobilis					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.5109	0.9778	0.3179	2.1161	0.9746	9.6801
Day 2	0.3014	0.5380	0.3148	1.9916	1.5701	8.2200
Day 3	0.4404	0.2657	0.2938	2.1496	0.9662	8.7948
Day 4	0.0012	0.0000	0.6144	0.4129	0.2182	1.3643
Day 5	0.4372	0.4023	0.3370	2.8606	0.8878	8.5379
Day 6	0.4799	0.5415	0.4522	2.9861	0.9117	9.0103
Day 7	0.4008	0.4113	0.2957	2.8393	0.9586	8.7762

	pH Amended Summary, Z. mobilis					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.5109	0.9778	0.3179	2.1161	0.9746	9.6801
Day 2	0.3699	0.5760	0.3237	2.0384	1.3514	8.3427
Day 3	0.5717	0.4759	0.3289	2.1917	1.1883	8.6387
Day 4	0.2149	0.6806	0.4949	1.0683	1.2287	3.5677
Day 5	0.4500	0.4358	0.2488	2.9543	0.8880	8.9515
Day 6	0.4557	0.5544	0.4845	2.9074	0.8785	9.3784
Day 7	0.4227	0.5010	0.4195	2.9050	0.9452	9.2416

	Yeast Extract Amended 1, Z. mobilis					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.6262	0.8362	0.2890	3.7242	0.9071	11.6270
Day 2	0.9473	0.6659	0.3510	2.6949	1.1977	13.2504
Day 3	0.4188	0.7532	0.2951	2.7374	1.1070	12.6443
Day 4	0.7776	0.6508	0.3229	2.7530	0.8981	11.9483
Day 5	0.5087	0.6594	0.2961	2.7543	0.8699	10.1285
Day 6	0.0000	0.7245	0.4270	3.0034	1.4625	11.1649
Day 7	0.8100	0.6190	0.4982	2.6575	0.8591	10.0507

	Yeast Extract Amended 2, Z. mobilis					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.6262	0.8362	0.2890	3.7242	0.9071	11.6270
Day 2	1.0551	0.7169	0.3637	2.7825	1.3067	13.4026
Day 3	0.4777	0.7633	0.3628	2.8337	1.0440	12.9031
Day 4	0.6208	0.6893	0.3590	2.7658	0.9061	12.1669
Day 5	0.0000	0.6265	0.3551	2.8244	1.1622	10.3297
Day 6	0.8011	0.7591	0.3912	3.0501	1.6509	10.9005
Day 7	0.8957	0.6908	0.3310	1.9679	1.0683	10.8234

	Yeast Extract Amended 3, Z. mobilis					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.6262	0.8362	0.2890	3.7242	0.9071	11.6270
Day 2	1.1518	0.7348	0.3369	2.8051	1.2449	12.9618
Day 3	0.5287	0.5858	0.2656	2.5634	0.8053	9.3435
Day 4	0.5653	0.7025	0.2989	2.7486	0.8042	11.4827
Day 5	0.6307	0.7036	0.3651	2.9361	0.5256	10.6359
Day 6	0.9216	0.6951	0.3658	2.6119	2.2194	11.2034
Day 7	0.8493	0.6803	0.2328	1.7858	1.5662	11.0012

	Yeast Extract Summary, Z. mobilis					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.6262	0.8362	0.2890	3.7242	0.9071	11.6270
Day 2	1.0514	0.7058	0.3506	2.7608	1.2498	13.2049
Day 3	0.4750	0.7008	0.3078	2.7115	0.9854	11.6303
Day 4	0.6546	0.6809	0.3269	2.7558	0.8695	11.8660
Day 5	0.3798	0.6631	0.3388	2.8383	0.8526	10.3647
Day 6	0.5742	0.7263	0.3947	2.8884	1.7776	11.0896
Day 7	0.8517	0.6634	0.3540	2.1371	1.1645	10.6251

	Unamended 1, C. shehatae					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.0000	0.8488	0.2660	3.7801	0.9756	11.3896
Day 2	0.0000	0.7820	0.4660	3.7527	1.0203	11.5638
Day 3	0.0000	0.7102	0.4249	3.5379	0.9954	11.6298
Day 4	0.6384	0.7401	0.4526	3.5606	1.1000	11.6532
Day 5	0.5369	0.7078	0.2477	3.6006	0.8125	10.9542
Day 6	0.4737	0.8123	0.4039	3.3280	0.8153	10.7095
Day 7	0.4725	0.8306	0.2936	2.4680	1.1028	11.4102

	Unamended 2, C. shehatae					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.0000	0.8488	0.2660	3.7801	0.9756	11.3896
Day 2	0.0000	0.7198	0.4907	3.5399	0.9984	11.9787
Day 3	0.5307	0.6722	0.4569	3.5320	1.0439	11.6895
Day 4	0.5446	0.7048	0.5025	3.5994	1.0210	12.9403
Day 5	0.5350	0.6823	0.4375	3.4395	0.7015	11.0937
Day 6	0.5445	0.8799	0.3832	3.1024	0.8300	11.0658
Day 7	0.5079	0.8312	0.2760	2.1989	1.0866	11.5887

	Unamended 3, C. shehatae					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.0000	0.8488	0.2660	3.7801	0.9756	11.3896
Day 2	0.0000	0.7239	0.4709	3.5440	0.9710	11.5948
Day 3	0.4537	0.6635	0.4568	3.6478	1.0366	11.0945
Day 4	0.0000	0.7856	0.9929	0.5040	4.1901	1.4801
Day 5	0.5689	0.7136	0.4109	3.4542	0.6551	10.7791
Day 6	0.4604	0.7860	0.3701	3.1063	0.8172	11.1064
Day 7	0.5792	0.8552	0.2797	2.0056	1.3005	11.8348

	Unamended Summary, C. shehatae					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.0000	0.8488	0.2660	3.7801	0.9756	11.3896
Day 2	0.0000	0.7419	0.4758	3.6122	0.9966	11.7125
Day 3	0.3281	0.6820	0.4462	3.5725	1.0253	11.4712
Day 4	0.3943	0.7435	0.6493	2.5547	2.1037	8.6912
Day 5	0.5469	0.7012	0.3654	3.4981	0.7230	10.9423
Day 6	0.4929	0.8261	0.3857	3.1789	0.8208	10.9606
Day 7	0.5199	0.8390	0.2831	2.2242	1.1633	11.6112

	pH Amended 1, C. shehatae					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.5109	0.9778	0.3179	2.1161	0.9746	9.6801
Day 2	0.4674	0.5718	0.3948	2.2986	1.5515	8.8515
Day 3	0.4169	0.5897	0.4186	2.4175	1.2996	9.0890
Day 4	0.5633	0.7694	0.4439	2.1084	0.9992	9.3247
Day 5	0.5388	0.8649	0.3258	2.1602	0.9882	9.4233
Day 6	0.5182	0.6060	0.4180	2.4487	1.3168	8.9993
Day 7	0.4183	0.5416	0.5440	3.1176	0.9410	9.5749

	pH Amended 2, C. shehatae					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.5109	0.9778	0.3179	2.1161	0.9746	9.6801
Day 2	0.4134	0.5982	0.3638	2.3254	1.5215	8.6872
Day 3	0.5259	0.6260	0.3498	2.4225	1.3114	8.9155
Day 4	0.3867	0.8579	0.5211	2.1457	1.0230	9.2549
Day 5	0.4768	0.8404	0.5741	2.1306	1.0113	9.7266
Day 6	0.0000	0.6561	0.3630	2.5006	1.3524	9.0555
Day 7	1.1556	0.9064	0.2463	3.2657	0.9316	9.4062

	pH Amended 3, C. shehatae					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.5109	0.9778	0.3179	2.1161	0.9746	9.6801
Day 2	0.3786	0.6219	0.4113	2.3851	1.4782	8.5506
Day 3	0.3654	0.5902	0.3846	2.4090	1.3575	8.9404
Day 4	0.5069	0.9029	0.5768	2.2239	1.0252	8.8120
Day 5	0.4565	0.8037	0.6235	2.0440	0.9050	9.0890
Day 6	0.4103	0.6225	0.3810	2.4681	1.2838	9.1006
Day 7	2.1314	1.7476	0.1738	4.3821	0.8236	9.4983

	pH Amended Summary, C. shehatae					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.5109	0.9778	0.3179	2.1161	0.9746	9.6801
Day 2	0.4198	0.5973	0.3900	2.3364	1.5171	8.6964
Day 3	0.4360	0.6020	0.3843	2.4163	1.3229	8.9816
Day 4	0.4856	0.8434	0.5139	2.1593	1.0158	9.1305
Day 5	0.4907	0.8363	0.5078	2.1116	0.9681	9.4130
Day 6	0.3095	0.6282	0.3874	2.4725	1.3177	9.0518
Day 7	1.2351	1.0652	0.3213	3.5885	0.8988	9.4931

	Yeast Extract Amended 1, C. shehatae					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.6262	0.8362	0.2890	3.7242	0.9071	11.6270
Day 2	0.6077	0.5660	0.4266	2.7189	1.2146	13.6197
Day 3	0.7401	0.8812	0.2146	2.8197	1.2714	13.7241
Day 4	0.5499	0.6823	0.6276	3.4855	0.9958	11.7440
Day 5	0.7307	0.6745	0.5896	3.8488	1.0735	11.8508
Day 6	0.0000	0.7245	0.4270	3.0034	1.4625	11.1649
Day 7	0.6878	0.7679	0.4277	2.2311	1.9142	11.6517

	Yeast Extract Amended 2, C. shehatae					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.6262	0.8362	0.2890	3.7242	0.9071	11.6270
Day 2	0.7218	0.7634	0.3460	2.8004	1.2537	13.4833
Day 3	0.7500	0.7040	0.3467	2.8235	1.2136	13.4696
Day 4	0.6429	0.7233	0.5849	3.6220	0.9801	11.7117
Day 5	0.6529	0.6777	0.5521	3.6139	0.8907	11.3438
Day 6	0.8011	0.7591	0.3912	3.0501	1.6509	10.9005
Day 7	0.7265	0.7694	0.2206	1.9142	1.6628	11.5630

	Yeast Extract Amended 3, C. shehatae					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.6262	0.8362	0.2890	3.7242	0.9071	11.6270
Day 2	0.7265	0.8360	0.4399	3.1751	1.4282	0.5249
Day 3	0.3871	0.7446	0.3058	2.7958	1.1737	13.3079
Day 4	0.8834	0.7362	0.6119	3.5577	1.0899	11.6974
Day 5	0.6174	0.6924	0.5267	3.6034	0.9187	11.3818
Day 6	0.0000	0.6941	0.3605	2.9695	1.7149	10.6279
Day 7	0.7313	0.7485	0.3541	2.0085	1.1804	11.5408

	Yeast Extract Amended Summary, C. shehatae					
	Cellobiose (mg/ml)	Maltose (mg/ml)	Glucose (mg/ml)	Xylose (mg/ml)	Galactose (mg/ml)	Mannose (mg/ml)
Day 1	0.6262	0.8362	0.2890	3.7242	0.9071	11.6270
Day 2	0.6854	0.7218	0.4042	2.8981	1.2988	9.2093
Day 3	0.6258	0.7766	0.2890	2.8130	1.2196	13.5005
Day 4	0.6921	0.7139	0.6081	3.5551	1.0219	11.7177
Day 5	0.6670	0.6815	0.5561	3.6887	0.9610	11.5255
Day 6	0.2670	0.7259	0.3929	3.0077	1.6094	10.8977
Day 7	0.7152	0.7619	0.3341	2.0513	1.5858	11.5852

APPENDIX C

GC Data and Procedures

Headspace Sampling for EtOH

GC Model:	SRI 8610C
Column:	MXT-1 (100% Dimethyl polysiloxane, non polar phase, Crossbond)
	Length = 60 m
	ID = 0.53 mm
	DF = 5.0 µm
Detectors:	PID/FID
	PID temp = 150 ºC
	FID temp = 140 ºC
Carrier gas:	He (ultra high purity) @ 30 psi (3 psi = 10 mL/min)
Air (zero grade):	7 psi (6 psi = 250 mL/min)
H2 (ultra high purity):	26 psi (26 psi = 25 mL/min)

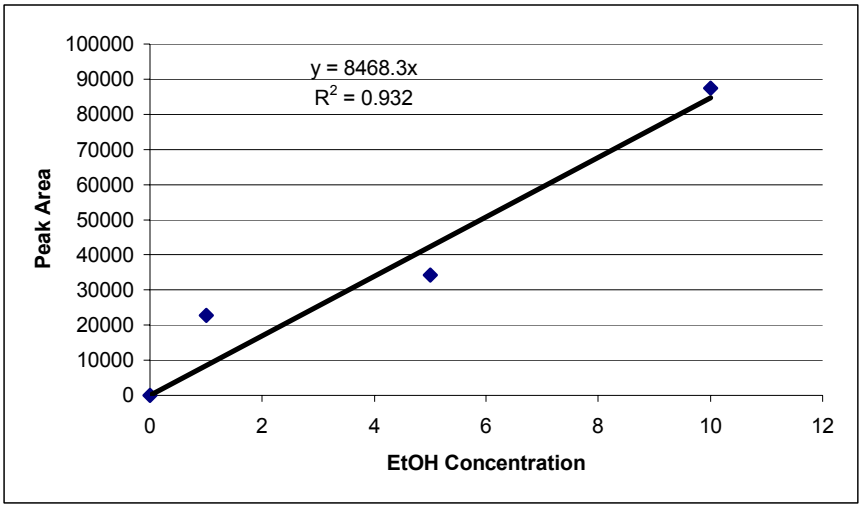
Temperature profile:

Initial temp:	40 ºC (5 min) ramp at 18 ºC/min
Final temp:	220 ºC (5 min)

Injection:	10 µL
Vol. of Vial:	20 mL
Sample Vol:	10 mL
Bath temp:	70 ºC

Calibration

Injection: 0.5 mL	Con'c (% v/v)	Retention (min)	Area
	0		0
	1	2.333	22793.922
	5	2.333	34153.129
	10	2.353	87344.423



		Yeast	Sugars		HSGC (03/09/07)				
Day 1	Sample	Con'c (% w/v)	% Brix	pH	Retention	Area	Height	Resolution	% EtOH
	0.5A1	0.5	9.6	4.35	2.316	25102.26	4586.5	3.833	2.9643
	0.5B1	0.5	9.6	4.36	2.283	13786.66	3232.4	4.182	1.6280
	0.5C1	0.5	9.6	4.37	2.300	31048.92	4991.6	3.286	3.6665
	1.0A1	1.0	9.7	4.47	2.300	38064.22	4990.4	0.714	4.4949
	1.0B1	1.0	9.7	4.46	2.400	42874.52	4831.7	0.677	5.0629
	1.0C1	1.0	9.7	4.45	2.316	17541.29	3148.7	3.462	2.0714
	1.5A1	1.5	9.9	4.53	2.350	15193.93	1804.5	3.000	1.7942
	1.5B1	1.5	9.9	4.50	2.316	12144.42	2565.8	0.634	1.4341
	1.5C1	1.5	10	4.52	2.366	31809.71	4004.1	3.462	3.7563
	CA1	control	9.3		2.316	6218.16	1120.1	0.643	0.7343
	CB1	control	9.3		2.300	6218.16	351.9	4.500	0.7343
	CC1	control	9.3		2.316	6218.16	1120.1	0.643	0.7343
			Sugars		HSGC (03/11/07)				
Day 2	Sample ID	Con'c (% w/v)	% Brix	pH	Retention	Area	Height	Resolution	% EtOH
	0.5A2	0.5	9.6	4.36	2.283	2793.90	476.9	0.354	0.3299
	0.5B2	0.5	9.6	4.31	2.416	3545.84	478.6	0.321	0.4187
	0.5C2	0.5	9.6	4.35	2.350	2651.15	326.2	1.625	0.3131
	1.0A2	1.0	9.6	4.44	2.400	4574.96	647.0	1.800	0.5402
	1.0B2	1.0	9.6	4.45	2.383	3036.61	468.0	2.000	0.3586
	1.0C2	1.0	9.7	4.43	2.383	3644.29	558.9	2.077	0.4303
	1.5A2	1.5	9.9	4.55	2.383	4628.07	625.2	1.688	0.5465
	1.5B2	1.5	9.9	4.50	2.350	3544.83	608.4	2.077	0.4186
	1.5C2	1.5	10	4.53	2.400	3405.46	445.2	1.625	0.4021
	CA2	control	9.3		2.350	2268.64	355.9	1.929	0.2679
	CB2	control	9.3		2.350	1965.36	271.8	3.067	0.2321
	CC2	control	9.3		2.366	2597.48	341.5	2.813	0.3067
			Sugars			HSGC			
Day 3	Sample ID	Con'c (% w/v)	% Brix	pH	Retention	Area	Height	Resolution	% EtOH
	0.5A3	0.5	9.5	4.33	2.400	2875.93	361.0	3.000	0.3396
	0.5B3	0.5	9.5	4.40	2.400	2875.93	361.0	3.000	0.3396
	0.5C3	0.5	9.5	4.43	2.416	3124.54	385.4	0.301	0.3690
	1.0A3	1.0	9.7	4.45	2.400	4596.63	622.3	0.591	0.5428
	1.0B3	1.0	9.7	4.50	2.383	5136.37	775.5	0.605	0.6065
	1.0C3	1.0	9.6	4.48	2.383	4402.99	586.3	0.565	0.5199
	1.5A3	1.5	10	4.58	2.433	8174.31	835.5	2.813	0.9653
	1.5B3	1.5	9.9	4.62	2.450	7446.39	816.5	2.933	0.8793
	1.5C3	1.5	9.9	4.55	2.383	5216.94	732.4	1.625	0.6161
	CA3	control	9.3	5.31	2.400	4891.31	700.2	3.214	0.5776
	CB3	control	9.2	5.29	2.466	4556.48	601.2	3.385	0.5381
	CC3	control	9.3	5.54	2.433	2232.39	255.9	0.533	0.2636
			Sugars			HSGC			
Day 4	Sample ID	Con'c (% w/v)	% Brix	pH	Retention	Area	Height	Resolution	% EtOH
	0.5A4	0.5	9.4	4.30	2.350	2383.03	374.4	2.077	0.2814
	0.5B4	0.5	9.5	4.30	2.500	3083.64	306.7	2.588	0.3641
	0.5C4	0.5	9.4	4.30	2.450	2804.51	338.4	3.462	0.3312
	1.0A4	1.0	9.8	4.40	2.433	4227.78	407.9	2.588	0.4992
	1.0B4	1.0	9.7	4.40	2.400	3253.54	491.5	1.929	0.3842
	1.0C4	1.0	9.8	4.40	2.366	2801.50	437.5	2.000	0.3308
	1.5A4	1.5	10	4.50	2.433	5167.12	749.1	1.929	0.6102
	1.5B4	1.5	10	4.50	2.183	2156.49	411.7	2.250	0.2547
	1.5C4	1.5	9.9	4.50	2.400	4146.87	563.4	0.614	0.4897
	CA4	control	9.3	5.61	2.400	1684.01	199.5	2.647	0.1989
	CB4	control	9.2	5.68	2.466	1542.27	211.0	3.667	0.1821
	CC4	control	9.2	5.74	2.433	625.65	109.8	0.667	0.0739
			Sugars			HSGC			
Day 5	Sample ID	Con'c (% w/v)	% Brix	pH	Retention	Area	Height	Resolution	% EtOH
	0.5A5	0.5	9.4	4.30	2.366	1348.29	204.8	2.077	0.1592
	0.5B5	0.5	9.4	4.33	2.366	1202.72	236.8	2.250	0.1420
	0.5C5	0.5	9.5	4.33	2.416	2581.23	340.5	1.857	0.3048
	1.0A5	1.0	9.7	4.40	2.333	2394.53	414.8	2.333	0.2828
	1.0B5	1.0	9.7	4.40	2.366	3235.26	467.9	1.800	0.3820
	1.0C5	1.0	9.6	4.39	2.383	4039.65	632.4	0.628	0.4770
	1.5A5	1.5	9.9	4.52	2.366	3289.46	475.2	1.688	0.3884
	1.5B5	1.5	9.9	4.52	2.400	4301.86	592.9	0.600	0.5080
	1.5C5	1.5	9.8	4.52	2.366	2254.62	484.9	2.455	0.2662
	CA5	control	9.2	5.61	2.383	2083.72	394.6	2.250	0.2461
	CB5	control	9.2	5.73	2.316	1342.71	288.3	2.250	0.1586
	CC5	control	9.2	5.77	2.300	735.56	179.1	2.333	0.0869

CURRICULUM VITA

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EDUCATION

Texas A&M University, College Station, TX — Expected Graduation: May 2007
Bachelor of Science in Biological Systems Engineering — GPA: 3.97/4.00

RESEARCH

Texas A&M University Undergraduate Research Fellows Program Class of 2007

- Thesis: *Conversion of Wood Molasses to Ethanol*
- Develop a research project to optimize the fermentation of wood molasses, an industrial waste stream, to ethanol
- Design a system for the industrial fermentation of the waste stream
- Skills: Gas chromatography, HPLC, microbe culture

Teaxas A&M Department of Plant Pathology and Microbiology—Starr Lab

- Student worker from October 2004 to August 2006
- Developed protocol for DNA identification techniques
- Conducted lab work in nematology research projects and laboratory upkeep
- Skills: PCR, agarose gel electrophoresis, DNA extraction, oil extraction

LEADERSHIP ACTIVITIES

L.T. Jordan Institute for International Awareness, *Research Fellows Director*
American Society of Agricultural Engineers, *Philanthropy Chair*
Tau Beta Pi, Engineering Honors Society, *Fundraising Chair*
National Association of Environmental Professionals, *Service Chair*

HONORS

Engineering Scholars Program	2006
Phi Kappa Phi Outstanding Junior for Texas A&M University	2006
Udall Honorable Mention, *National Scholarship in Environmental Policy*	2005
Alpha Epsilon, *Honors Society of Biological and Agricultural Engineers*	2005
Gamma Sigma Delta, *Honors Society of Agriculture*	2005
University Scholar	2004

WORK EXPERIENCE

Texas A&M University Industrial Assessment Center, Texas A&M
IAC Team Member — August 2005 – Present

- Visit manufacturing plants across Texas to analyze their energy use and efficiency
- Recommend improvements to cost and energy efficiency based on assessments

Natural Resources Conservation Service, USDA, Corsicana, TX
Intern — May 2004 – August 2004
Assisted with engineering projects and surveying

Department of Biological and Agricultural Engineering, Texas A&M
Student Technician — February 2004 – May 2004
Cataloged papers and books