DDB 1111


# Analyzing the Ability of Modified Yeast to Ferment Xylose to Ethanol

An attempt to increase the ethanol yield from the fermentation of pentoses for the purposes of fuel use.

A Major Qualifying Project submitted to the faculty of Worcester Polytechnic Institute in partial fulfillment of the requirements for the Degree of Bachelor of Science

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## Abstract

Ethanol fermented from biomass is a significant alternative fuel source, but the current process of fermenting corn is not sustainable. Lignocellulose has been considered an alternative biomass, yet difficulties remain in breaking down the material and fully fermenting it. One major obstacle is the presence of xylose and other pentose sugars which are not fermented by the standard microorganism used, *S. cerevisiae*. This project studied a modified strain of yeast and its ability to ferment xylose. It was determined that the modified yeast is capable of using xylose as long as some glucose is also present. The yeast performed optimally when greater than 50% of the substrate used was glucose. Additionally, it was found that the modified yeast grew at half the rate that the wild yeast did.

## Acknowledgments

The team would like to thank advisor Professor David DiBiasio, and co-advisor Professor William Clark. In addition, specials thanks is given to Professor Reeta Prusty Rao and graduate student Jeff Swana for allowing the team to work with their modified strain of yeast.

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## Executive Summary

America's dependence on foreign fossil fuels has been at the front of newspaper headlines and political discussions for many years. In addition, the environmental effects of such wide spread use of fossil fuels has encouraged many to seek alternative resources. Liquid fuels are particularly important in the transportation industry, and one promising alternative is ethanol fermented from biomass. The biomass can be grown on American soil, reducing foreign dependence. Another benefit is that the net carbon emissions from the combustion of this ethanol is zero, since the source of the carbon is biomass. These two critical factors have given great weight to value of this fuel. The main argument against the current ethanol fermentation process is the use of corn as the feedstock. Corn is a staple in the American food industry and an increased demand for corn has many worried that the food source will become strained. Additionally, the energy required to produce ethanol from corn is much greater than the energy in fuel.

One alternative to corn is lignocellulose. Lignocellulose is a complex compound containing lignin, cellulose, and hemicellulose. The current problems surrounding the use of this material are twofold. First, the lignin is not useable in the fermentation process, so the cellulose must be separated from it. Lignin, however, is tough and resistant to chemical and biological processes to break it down. Better forms of hydrolysis, the process that breaks down this complex structure into basic sugars, are required before the alternative can be economically viable. The second problem surrounds the sugars that are produced during hydrolysis. Corn is made up almost entirely of glucose, a six carbon chain sugar. *S. cerevisiae* is a strain of yeast commonly used to ferment glucose into ethanol, as it ferments this sugar efficiently and quickly. However, unlike corn, cellulose is comprised of both glucose and xylose, a five carbon chain sugar. *S. cerevisiae* cannot metabolize xylose, and therefore a substantial amount of available material is not converted to ethanol and the fuel yield is decreased. This decreases the ratio of energy returned to energy put into the process, so much that the process is no longer considered a viable solution. If the xylose could also be fermented to ethanol the process has the ability to provide a sustainable energy source for The United States.

The strain of *S. cerevisiae* studied in this report was genetically altered to allow it to metabolize xylose as well as glucose. A total of nine genes were added from a different microorganism that can ferment xylose, but cannot survive in an ethanol environment. In addition, an inhibitory gene found in *S. cerevisiae* was deleted. The ability of this modified strain of yeast to ferment xylose and a

combination of glucose and xylose was observed. In order to do so, both the wild type and the modified yeast were grown in media containing various ratios of glucose and xylose. The growth rate of each type of yeast and the ethanol concentration were monitored over time and compared.

It was first discovered that the modified yeast had a slower growth rate than the wild type yeast. While this is not necessarily an issue, it does mean that the rate of ethanol production is slower, which could impact an industrial process. It was also noted that the modified yeast does not use glucose as efficiently as wild type yeast. This is expected, as the metabolic pathway of this yeast has been altered. The modified yeast did successfully ferment xylose, a finding confirmed by material balances. When a 60/40 glucose to xylose ratio was used, the modified yeast performed much greater than the wild type yeast in ethanol production.

It was also determined that the modified yeast required some of the substrate to be glucose. This was expected because glucose is the preferred sugar for cell growth in most organisms, as this is a very energy intensive process. When grown on only xylose, both the wild type yeast and the recombinant strain failed to produce any ethanol. The strains were also compared under very low concentrations of glucose; both performed similarly. It was not until the glucose concentration reached 60 percent that the modified yeast performed better than the wild type yeast. However, as many types of cellulosic material contain ratios similar to this, this is not a foreseeable problem.

The performance of this strain of yeast was only compared under one type of reactor conditions. Altering the temperature, pH, and other variables may improve the performance and ethanol yield. Additionally, only xylose was studied in this experiment, though other pentose sugars such as arabinose are also present in the lignocelluloses material. It would be important to study the recombinant strain's ability to ferment these other sugars, as their conversion to ethanol increases the overall yield. A separate study regarding the hydrolysis of the lignocelluloses material is also required before the process can become a sustainable energy option.

# Introduction

Biofuels have the potential to be fully integrated within the U.S energy portfolio and can thus enable the goals for energy security and greenhouse gas reduction to be achieved. [1] America's national security, economy, and environment are at great risk due to a high dependence on fossil fuels. [2] The United States consumes twenty five percent of the world's total oil production. [2] Additionally, tens of billions of dollars are spent annually in order to import oil from unstable regions of the world. [2] Thus, energy needs are prominent and biofuels can be used to amend the existent national reliance on oil. [2]

Fuels that are made from living organisms or from metabolic by-products such as organic or food wastes are known as biofuels. [4] Biofuels are energy carriers which store energy drawn from biomass. [6] Some forms of biomass and their respective sources includes food, fiber, and wood process residues from the industrial sector, in addition to energy crops, short-rotation crops, agricultural waste, and residues from the forestry sector. [6] These varying forms of biomass can be used to produce electricity, heat, and other forms of bioenergy.

There must be greater than eighty percent of renewable materials within a fuel in order for it to be considered a biofuel. [4] Renewable energy comes from resources that are replaced at a rate comparable to the consumption rate, and thus have sustainable benefits over fossil fuels such as petroleum and coal. [4] Additionally, biofuels are considered carbon neutral due to the fact that the carbon released during combustion is equal to the carbon captured by the biomass during the plants' life cycle. [5]

Biomass has been used to generate biofuels in the United States since the beginning of the twentieth century. Interest in them increased dramatically as a result of the fuel crisis in the 1970's; however, after the oil market stabilized, research funding sharply declined. Recently, the production of ethanol for the purposes of biofuels has greatly increased due to the diminishing supply of fossil fuels, and this has had an immense impact on U.S agriculture. [7] More than two billion bushels of corn annually are being used for ethanol production. Due to this large quantity for the purposes of biofuel production, a prevailing "food versus fuel" debate has come about. [7]

Due to the increasing demand for renewable fuels, there is a great deal of pressure on the agricultural sector since both the food processors and biofuel producers must compete for the same crop. [8] Various raw materials used in the production of biofuels such as corn, sugarcane, and soybeans are a source of food for millions of people. [8] As a result of this conflicting demand of both industries,

the food versus fuel debate has surfaced and includes an argument as to whether or not people will go hungry and a food shortage will result due to the high demand for biofuels. [7] There is a dispute between ethanol producers and the food industry that claims food costs will substantially rise and crop availability will decrease due to the increasingly high use of corn for biofuel production. [10]

The main argument of the food industries that is used against ethanol producers includes the fact that corn, which could feed the hungry, is instead being used to produce ethanol. [7] However, on the contrary, a minimal volume of U.S corn is exported to the undernourished populations around the world. [7] Information collected by the Food and Agricultural Organization of the United Nations (FAO) reports that ten countries with the highest percentile of undernourished populations, only receive less than .01 percent of U.S corn exports. By contrast, fifty five percent of U.S corn exports are sent to wealthy nations in the Organization for Economic Cooperation and Development (OECD). [7]

On the other hand, there is a real concern that biofuel production will have a negative impact in countries with high levels of food insecurity. The high demand for biofuels in wealthy countries could hinder national food production. [7]

Food companies claim that the rising corn prices are a result of the high demand for ethanol; therefore, they must raise the prices of corn-containing products and other meat and dairy products obtained from grain-fed animals. [10] A spokeswoman for the American Pop Corn Company, Tracy Boever, argues that although the company is not holding anyone responsible for the higher prices, "the fact remains that there are only so many acres of land and the popcorn industry, along with others, are competing for those acres."[1] Thus, there exists a competition for corn between ethanol and food industries, who feels that it is being negatively impacted by ethanol production.

From a political standpoint, Democrats, Republicans, the White House, and U.S Congress all support an immense expansion of the biofuel industry. On January 23, 2009, President George W. Bush called for 35 billion gallons of renewable fuels annually in his State of the Union address. This amount translates to an equivalent of fifteen percent of gasoline burned in cars and trucks. Furthermore, the U.S. Congress is contemplating measures that would call for sixty billion gallons of renewable fuel by the year 2030. [9] This number is significant, showing the importance of research into renewable resources such as bioethanol.

Bioethanol production is possible from low cost materials including lignocellulose and other agricultural remains. Agricultural remains include corn stover, wheat straw, and rice straw, and processing by-products such as corn fiber, rice hulls, and sugarcane bagasse. [11] Additionally, specific

[1] [10]

energy crops can be grown. One example is switch grass, a low costing sugar source that can be implemented towards the production of biofuels. Lignocellulosic biomass is then converted to fermentable sugars for the production of biofuels; however, many problems currently exist both on a technical and economic level. [11]

In 2006, corn grain-based ethanol production was approximately 5 billion gallons; in relation, a total of 140 billion gallons of transportation fuel is used annually. [11] The long term goal is to replace about thirty percent of the national motor gasoline use with ethanol by the year 2030; this requires production levels of approximately 60 billion gallons on an annual basis. Thus, it is essential that ethanol production is promoted by the development of lignocellulose such as feedstock due to the great quantity in which it can be found. [11]

Lignocellulose consists of two main sugars: glucose and xylose. [13] Glucose, which is a six carbon sugar, is commonly referred to as blood sugar. [17] It is currently used in many areas, including food production. Xylose, a five carbon wood sugar, is not utilized currently, but is found in significant quantities in lignocellulose. [15] Industries such as paper production leave leftover plant matter containing xylose, without any current use for it. [13] This makes it an economically viable sugar to consider as an alternative to glucose.

Research is needed for the development of utilizing five carbon sugars for the production of ethanol. Once adequate research has been done, a process can be streamlined for the mass production of ethanol, thus ultimately alleviating our national dependence on oil. This MQP team plans to conduct research in this field, specifically, for the investigation of producing ethanol from xylose.

In order to determine whether or not xylose can be employed for the production of ethanol, four strains of yeast obtained from the laboratory of Professor Reeta Prusty Rao at Worcester Polytechnic Institute were examined. Because *S. cerevisiae* (S288C), a common strain of yeast referred to as Baker's yeast, does not naturally have the ability to ferment xylose, three yeast strains have been genetically modified and compared to the wild type. One of the strains has a lignocellulosic transgenic cassette (LTC) containing a collection of nine inserted genes (see Table 1), the second simply has the Gre3 gene deleted, and lastly, the third contains the LTC additions and omits the Gre3 gene. The production of ethanol from varying amounts of xylose and glucose by the four strains of yeast were quantitatively determined and compared. The amount of ethanol that was produced by each strain within these laboratory conditions was determined, and recommendations for further study were discussed.

Small-scale research for the production of ethanol from xylose will contribute to the overall, large scale research demand and as a result, the potential for a streamlined process on an industrial scale for the production of biofuels can be evaluated. Variables in the bioreactor include temperature, pH, mixing rate, and substrate and protein concentrations. A higher temperature can increase the rate of the reaction; however it cannot exceed the temperature in which yeast can survive. As with most biological processes, the reaction will only move forward in a specific pH range, though this microorganism naturally creates the proper pH environment. A well-mixed reactor allows yeast to come in contact with substrate, increasing the rate of the reaction. It is necessary for excess proteins to be provided to ensure proper cell growth, allowing for complete substrate fermentation. By conducting such research, the potential to expand this into an economically viable and efficient industrial process holds promise.

## Background

Biomass goes through a complex four-part process to generate ethanol, which is used as a fuel in place of or in addition to conventional petroleum products. Raw materials, both grain and cellulosic biomass, are first pretreated in order to begin breaking down the material and generating more surface area for the second step, hydrolysis. Hydrolysis is accomplished either by the use of enzymes or chemicals. In this step the complex carbohydrate chains in the biomass are broken down to simple sugars. Finally, these sugars are fermented by microorganisms, either yeast, fungi, or bacteria, which produce ethanol in a dilute form. In order to concentrate the ethanol, distillation techniques are used. If pure ethanol is required, the product is subjected to further separation techniques.

Due to previously stated reasons, the use of corn grain to produce fuel is not ideal. Rather, it is desirable to use agricultural waste such as corn stover or other woody waste, that has no other practical uses; however, this biomass is cellulosic and a more complex polymer than the starch in corn kernels.

### Pretreatment and Hydrolysis

Currently, the biomass used in the United States is starch derived from corn grains. There are two pretreatment processes that corn can go through in order for it to be fermented: wet milling and dry milling.

The process of wet milling requires the corn kernels to be soaked in a dilute acid prior to processing in order to begin breaking down the grain into its individual components such as starch, protein, germ, and oil. [22] In dry milling the corn kernels are ground into a fine powder called meal. In both circumstances enzymes are then used to break down the long carbohydrate chains into shorter glucose chains that can be fermented. This step is referred to as saccharification. [22] Next, these simple glucose sugars are fermented by microorganisms, generally yeast. The yeast feeds and grows off of the glucose that is generated by hydrolysis and ultimately produces ethanol, as shown in the mechanism in Figure 1 below.

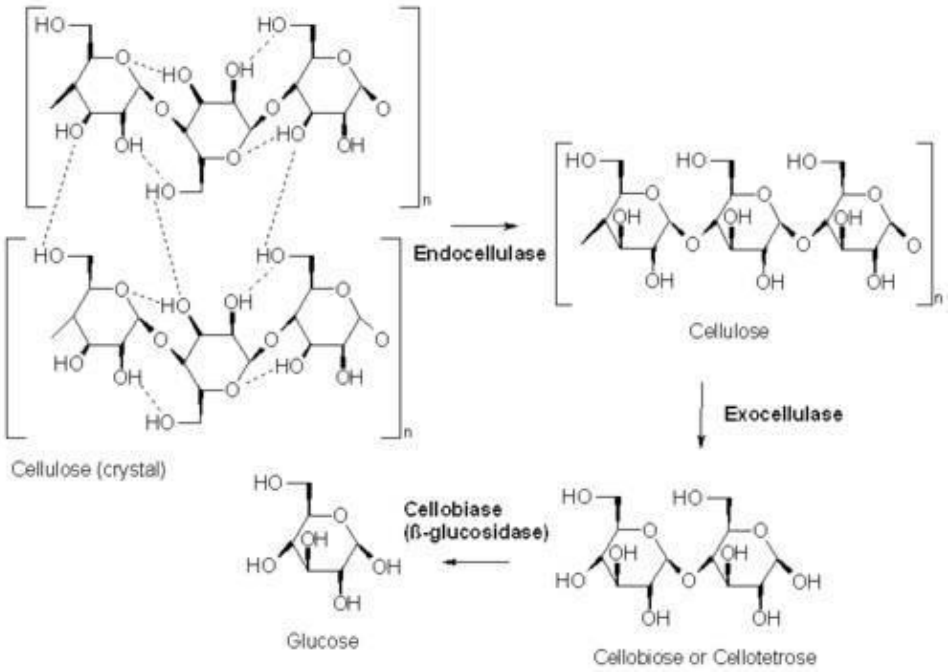


**Figure 1: Reaction Pathway of Cellulose into Glucose. Provided by Reference Answers.**

Lignocellulosic biomass is comprised of various agricultural residues, deciduous and coniferous woods, municipal solid wastes, waste from pulp and the paper industry, and various energy crops. These materials primarily contain cellulose (35 to 50%). Other fractions include 20 to 35% hemicellulose and 10 to 25% lignin. [11] The sugars found in lignocellulose are primarily existent in two forms:  pentose and hexose. [13]

Lignins can be found in the cell walls of woody species and are highly branched, substituted, mononuclear aromatic polymers, as seen in Figure 2 below.

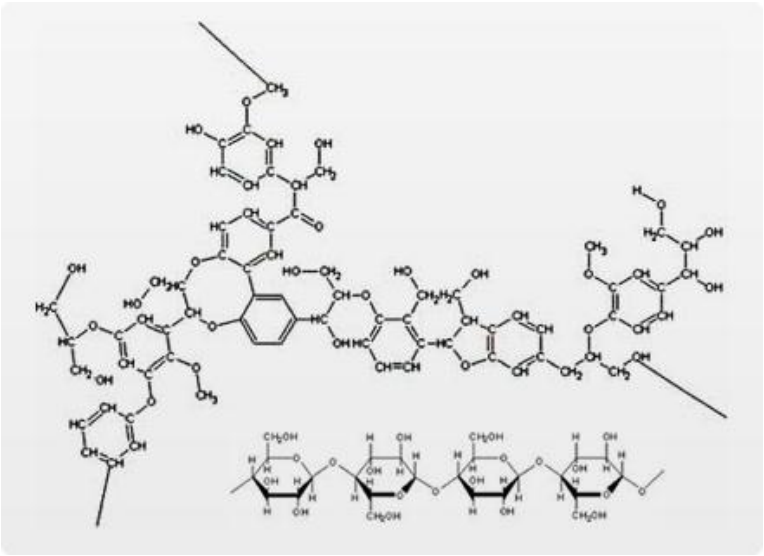

**Figure 2: Molecular structure of lignocelluloses. Provided by Electregy Revolution Coproation.**

Lignin found in biomass tends to bind to surrounding cellulose fibers and form a lignocellulosic complex. The lignin surrounds the more usable hemicellulose and cellulose material and is difficult to break into, see Figure 3.

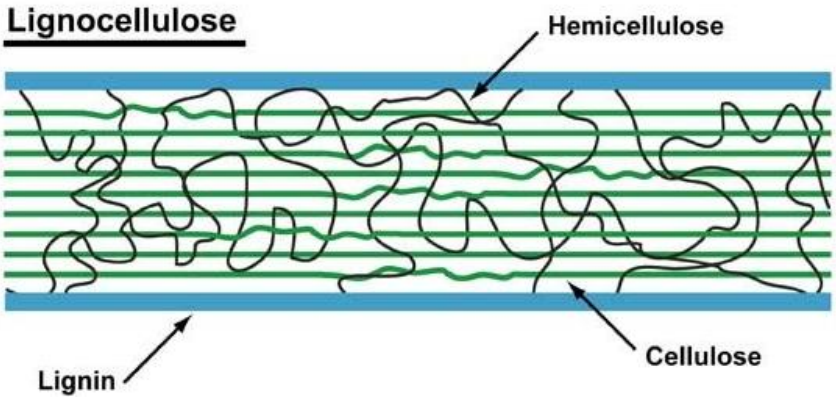


**Figure 3: Lignin surrounds hemicelluloses and cellulose in a plant cell wall. Provided by The International FEW Biomass Magazine.**

Similar to corn, a two-step process is needed to break down the biomass before the sugars can be used for fermentation. A broad overview of this process can be seen in Figure 4 below. The lignocellulosic complex is resistant to microbial systems and chemical agents, making it difficult to break down. [12]

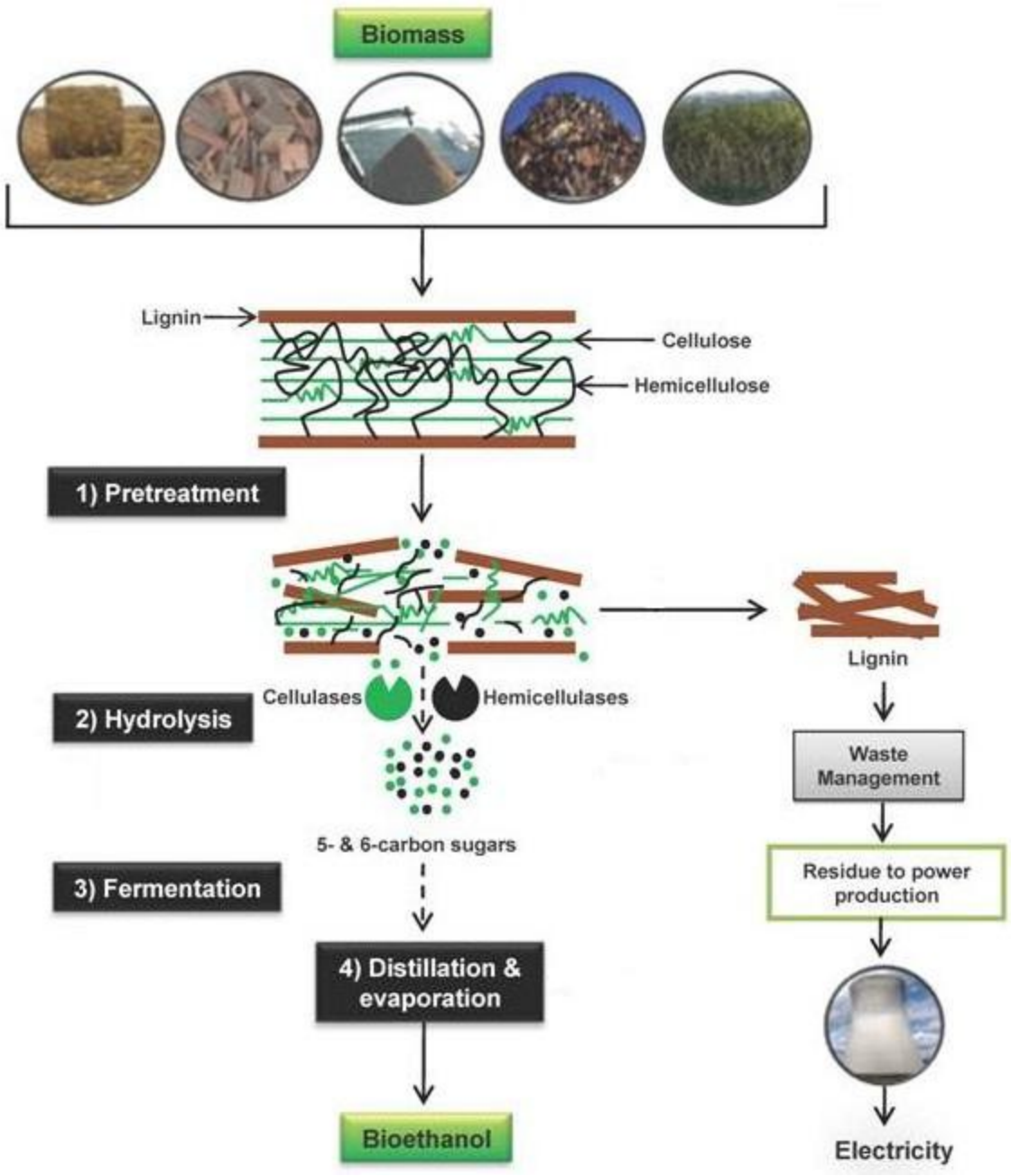


**Figure 4: An overview of the process from lignocellulose to biofuels. Provided by the International Journal of Biological Studies.**

Various forms of pretreatment are used to begin the breakdown of the cellulose. These pretreatments include strong acids such as sulfuric acid, oxidation, steam explosion, ammonia fiber explosion, $CO_2$ explosion, liquid hot water, and organic solvent treatments. [11] This decreases the crystallinity, therefore increasing the surface area on which hydrolysis can occur. [11] Many of the pretreatment methods that are used generate soluble and only partially degraded hemicellulosic biomass, therefore hydrolysis is also needed in order to produce fermentable sugars. [11]

Hydrolosis can be accomplished by the use of enzymes or chemicals to further decompose starch or cellulose to simple sugars. [23] Enzymatic hydrolysis is performed by the use of the enzymes endo- 1,4-Beta-glucanase, exo- 1,2-Beta-glucanase, and B-glucosidase. [11] The step of cellulose hydrolysis is a prominent element of the total production cost of ethanol from lignocellulosic material. [11] Alternatively, chemical hydrolysis can be used. Acid hydrolysis is becoming more popular due to its lower cost and greater effectiveness then enzymatic hydrolysis. [24] The lignocellulosic material is subjected to strong concentrations of hydrochloric or sulfuric acid in order to begin the break down and separation of materials. [24]

There are currently many economical and technical problems associated with the conversion of lignocellulosic biomass to fermentable sugars. [11] In order to successfully enable fermentation, it would be essential to develop an effective pretreatment of the lignocellulose and have efficient enzyme conversion to fermentable sugars. [11]

## Sugars Used in Ethanol Fermentation

Ethanol fermentation is performed by converting sugars to make ethanol and carbon dioxide. [14] Other byproducts can also be produced, depending on the sugars that are present and the microorganisms that are used for fermentation. The most common type of sugar found in corn is hexose, and both pentose and hexose are found in significant quantities in lignocellulosic material. [14]

Pentose is a monosaccharide, or simple sugar, with five carbon atoms. [15] A large percentage of the sugars found in lignocellulose is xylose, or wood sugar, which is composed of a pentose with an aldehyde functional group. [16] Xylose and arabinose, another aldopentose, is found primarily in the hemicellulosic part of the plant. It is frequently found as a waste product of the papermaking industry, thus making it desirable as an economic energy source for ethanol production. [16]

Hexose is a monosaccharide like pentose, however it contains six carbon atoms. [17] Glucose is a common form of hexose containing an aldehyde group, just as xylose. Two stereoisomers, L-glucose and D-glucose, exist. Only D-glucose, or dextrose, is found in nature however. It is also known as blood sugar or corn sugar and is found in the cellulosic part of biomass along with smaller amounts of mannose, galactose, and rhamnose. Glucose is readily available and is a major energy source for most organisms, including humans, which leads to the concern of both the cost and use of valuable food resources. [17]

## Microorganisms Used in Cellulosic Ethanol Fermentation

The microorganisms used for fermenting ethanol can be yeast or bacteria. Each has its strengths and weaknesses in their abilities to ferment different types of sugars. Bacteria are a prokaryotic, meaning they lack the cell nuclei that can be found in eukaryotic cells such as yeast. [30] There are several strains of bacteria that are used in ethanol fermentation and they are distinguished by the sugars they ferment and the products that result from fermentation.

Clostridium thermocellum can successfully convert cellulose directly into ethanol without needing a saccarification step beforehand. [30] However, the downside to using this bacterium is that it produces a low ethanol yield due to the production of byproducts such as lactate, acetate, and formate. Zymomonas mobilis is another type of bacteria that surpasses yeast in ethanol yield and tolerance. [31] Neither bacterium however is able to naturally ferment xylose although, a variant of *Z. mobilis* that can ferment certain pentoses has been developed. Unfortunately, this bacterium has a lower tolerance than yeast to inhibitory compounds produced during the hydrolosis of lignocellulose; this results in a lower ethanol yield on an industrial scale. [31]

Yeast is currently the most popular method for converting cellulosic sugars into ethanol. The most common type of yeast used is Saccharomyces cerevisiae, also known as Brewer's yeast or Baker's yeast. [19] *S. cerevisiae* is a popular choice because it has a relatively high tolerance to ethanol and inhibitor compounds and furthermore, can produce high ethanol yields from glucose. Similar to most bacteria, this yeast is unable to ferment xylose and other pentose sugars at this time. [19] Pichia stipitis is another yeast in the same family as Brewer's yeast that has the highest ability of any yeast to directly ferment xylose into ethanol. [20] Additionally, it is capable of both aerobic and anaerobic fermentation, meaning it is able to do so both with and without the presence of oxygen. *S. cerevisiae*, on the other hand, can only ferment in an anaerobic environment. In spite of this, the downside to *P. Stipitis* is its low tolerance to ethanol that results in restrictions to industrialization of the process.

### Xylose and Pentose Utilization

*S. cerevisiae* is preferred for fermentation because of its high tolerance to ethanol in comparison to other microorganisms. [25] In addition, it is highly efficient at metabolizing hexose sugars like glucose; however, its use of pentose is restricted. [11] New strains of yeast are essential to meet the needs of the biofuels industry, especially due to the fact that *S. cerevisiae* is incapable of utilizing xylose. This underlying challenge poses great limits and boundaries upon which biomass can be used to produce ethanol from xylose found in inexpensive, economically viable feedstock. [11]

Within the past two decades, there have been advances in the use of xylose for ethanol production by means of *S. cerevisiae*. [11] Naturally, this strain of yeast does not utilize xylose, however, it contains a pathway that is capable of consuming xylose in the presence of D-ribose and other substrates. In eukaryotes, which include yeast and other fungi, the conversion of xylose occurs by means of two metabolic pathways. The typical pathway, referred to as the oxo-reductive pathway, is xylose reduction to xylitol, and xylitol oxidation to xylulose. [26] The conversion of xylose into xylitol uses most of the available NADP+, an oxidizing agent which accepts electrons, and NADH, a reducing agent which can donate electrons. [11]These enzymes are also needed to oxidize xylitol into xylulose. [11] As a result, there is little xylulose to enable ethanol production. [26] The second pathway is similar to the way bacteria metabolize xylose. In this pathway, the isomerase pathway, the xylose is converted directly into xylulose. This allows for an increased production of ethanol. [26] A comparison of the two pathways can be seen in Figure 5.

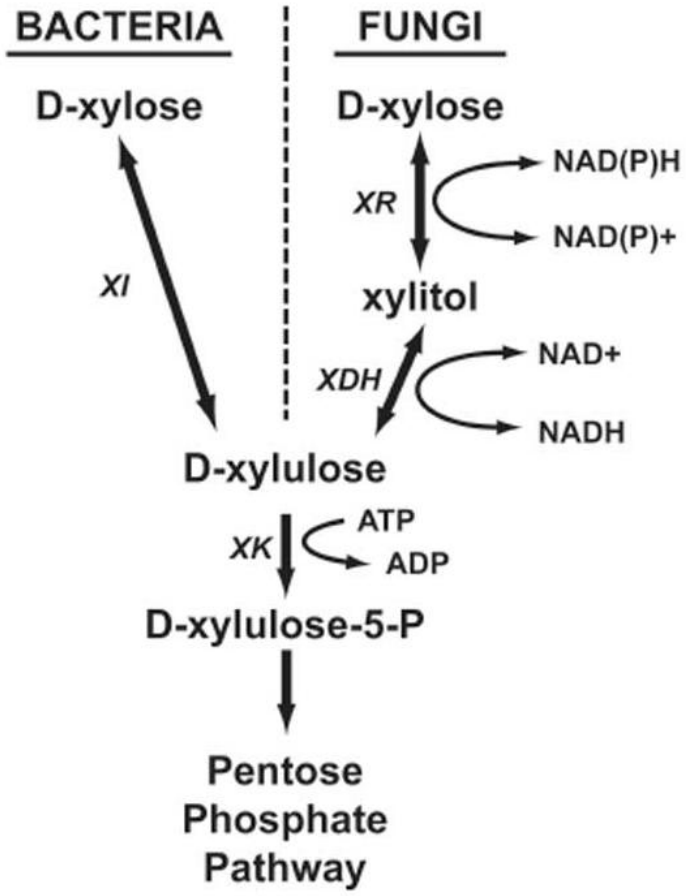


**Figure 5: A comparison of the two metabolic pathways. Provided by The International FEW Biomass Magazine.**

## Recombinant S. cerevisiae for Xylose Fermentation

Current research is examining several ways to increase the efficiency of processing five carbon sugars found in cellulosic biomass. One solution to this problem is being developed by Stephen Hughes at the ARS National Center for Agricultural Utilization Research in Illinois. [27] He has developed a new strain of yeast where xylose is used by the yeast as the required substrate to grow and reproduce, without oxygen. Because the xylose is supporting the growth of the yeast, all of the glucose present can be converted into ethanol. [27] In 1993, the first genetically modified yeast strain to ferment both kinds of sugars was produced, but the fermentation rates remained low. [28]

Several universities, including Worcester Polytechnic Institute, have researched genetically modifying *S. cerevisiae* so that it can ferment xylose into ethanol at higher fermentation rates. [29] Two genes found in *P. stipitis*, XYL1 and XYL2, were introduced to *S. cerevisiae*, as well as xylose isomerase, which is an enzyme that catalyzes the metabolization of xylose. An endogenous gene in *S. cerevisiae* that is involved in the pentose phosphate pathway was up-regulated. The combination of the genes and others is known as the lignocellulosic transgenic cassette, or LTC, see Table 1.

**Table 1: Summary of LTC genes**

| Gene | Source Organism | Protein | Purpose |
|---|---|---|---|
| Xyl1 | Pichia stipitis | Xylose Reductase (XR) | Reduces D-xylose to xylitol. |
| Xyl2 | Pichia stipitis | Xylulose reductase/Xylitol dehydrogenase | Oxidizes xylitol to D-xylulose |
| Xyl3 (XKS1) | Pichia stipitis | Xylulokinase | Converts D-xylulose to D-xylulose-5-P |
| XylA | Streptomyces diastaticus | Xylose Isomerase | Converts D-xylose to D-xylulose |
| Tal1 | Saccharomyces cerevisiae | Transaldolase | Increase flux through the pentose phosphate pathway |
| Stl1 | Pichia stipitis | Sugar Transporter Like protein | Xylose uptake |
| AraA | Salmonella typhimurium | L-arabinose isomerase | Converts L-arabinose to L-ribulose |
| AraB | Salmonella typhimurium | L-ribulokinase | Converts L-ribulose to L-ribulose-5-P |
| AraD | Bacillus subtilis | L-ribulose-5-phosphate 4-epimerase | Converts L-ribulose-5-P to D-xylulose-5-P |

Together, they increase the expression of the genes that allow the yeast to convert the sugar into an intermediate that can be fed into glycolysis. Lastly, an aldose reductase found in *S. cerevisiae*,

referred to as Gre3, was removed, as this enzyme inhibits the process. The Gre3 gene is involved in the production of xylitol, which as discussed previously is detrimental to the process. This recombinant yeast strain should allow for the fermentation of both pentose and hexose and therefore result in a higher ethanol yield. [29]

# Methodology

In these experiments, four different strains of yeast and their ability to utilize xylose were investigated. Specifically, the strains of yeast that were studied include: a wild-type *S. cerevisiae* (S288C), the wild-type with a collection of nine genes that have been added, a wild-type with a Gre3 gene deletion, and lastly the strain with both the nine genes added as well as the deletion of the Gre3 gene. This collection of nine genes is involved in xylose and arabinose metabolism and is referred to as the lignocellulosic transgenic cassette (LTC).

Four runs were conducted in these experimentations over a period of approximately two months, with each individual run containing varying substrate (sugar) concentrations. The purpose was to determine the trend that exists when the different strains of yeast utilize the varying amounts of glucose and xylose that it is supplied to them.

## Preparation of Media

The media was prepared by mixing 1 liter of water with 10 grams of bacto-peptone and 5 grams of yeast extract. While 20 grams of sugar was added to this mixture in the first run, thereafter 10 grams of sugar was added instead to allow for faster fermentation times. The ratio of glucose to xylose was varied in each experimental run. 250 ml of the media was then transferred into each of six shake flasks labeled A-F and capped with a sponge top. Media containing 10 g/L of glucose was prepared to use as an inoculation media. All media was autoclaved for 15 minutes immediately following its preparation in order to sterilize it and insure that no other microorganisms were present during fermentation.

## Inoculation

5 ml of the inoculation media was transferred into each of six culture tubes that had previously been autoclaved and labeled A-F corresponding to the respective shake flasks. One to two colonies of yeast were added to each tube, and capped with a sponge top. The colonies were transferred from the petri dish to the liquid media using sterilized toothpicks. These culture tubes were then placed in the shake bath and maintained at a temperature of 31±1°C for 48±4 hours. This process allowed the colonies to begin growing, with the assumption that the culture tubes reached full saturation at 48 hours, meaning no more cells could grow in that media.

After 48 hours, the contents of each culture tube were transferred via a pipette to the respective 250ml shake flask. At this time, time zero samples were taken and the flasks were placed in the shake bath and maintained at 31±1°C. The shake speed was set so that the mixing was visible. The flasks remained in the shaker for the duration of the run, about 48 hours.

## Measuring Cell Count and Ethanol Concentration

Roughly every one to two hours a 1ml sample was taken from each flask to determine cell density and a 3 ml sample was taken to determine ethanol content. In order to determine cell density a Milton Roy Spectronic 20D instrument was used with a tungsten lamp at a wavelength of 600 nm. Before measuring the samples, the concentration range where a linear relationship existed between concentration and absorbance was determined. A culture tube with inoculation media and a colony of wild type yeast was allowed to come to full saturation. This was then diluted with deionized water to various known concentrations, assuming the full saturation to be a concentration of 1. Each dilution was measured in the Spec 20D and the absorbance read. The concentration was plotted against the absorbance and the linear range was noted. As seen in Figure 6 below, absorbance readings below 0.250 comply with Beer's Law and maintain a linear relationship with concentration.

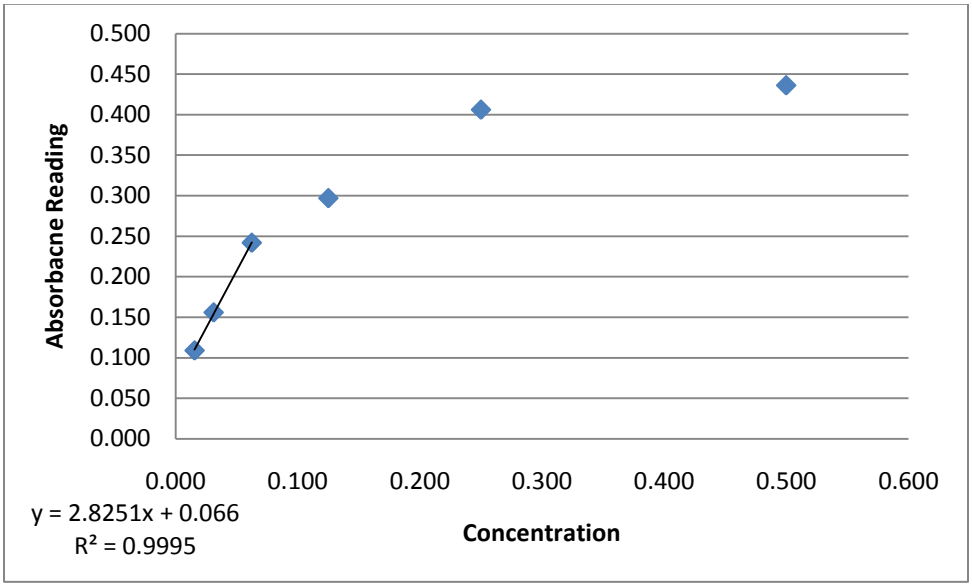


Figure 6: Spec 20D Calibration Curve

Each sample taken from the shake flasks were placed in a clean glass test tube and diluted with deionized water until the Spec 20D reading was close to or below 0.250. Before each use the instrument was zeroed with deionized water. A minimum of 3 ml was required to be in the test tube for an accurate reading, so the minimum dilution used was 1 ml sample to 3 ml total. The dilution factor and absorbance reading were noted. A corrected absorbance was determined by multiplying the actual reading by the dilution ratio, this was then plotted against time.

The 3 ml samples taken to determine ethanol concentration were first centrifuged until the solids fell to the bottom and the solution became transparent. A pipette was used to transfer the transparent solution off of the top, which was then filtered using 0.2 nm syringe filters and placed in a 4

ml drum vial and sealed. ½ ml of the filtered solution was diluted with 24.5 ml of water for a 1/50 dilution ratio before being run through a Perkin Elmer gas chromatograph. This dilution was done to reduce the chance of any residual cell particles left in the sample entering the GC column. Every sample was run through the GC twice and an average area was calculated.

In order to translate the peak area reading from the GC into an ethanol concentration several standards of known ethanol concentrations were prepared and diluted 1/50. The peak areas were then plotted against the known concentrations. As seen in Figure 7 below, the region below 3% ethanol maintains a linear relationship. All of the samples from the experiments were found to be less than 3%, thus this relationship is valid.

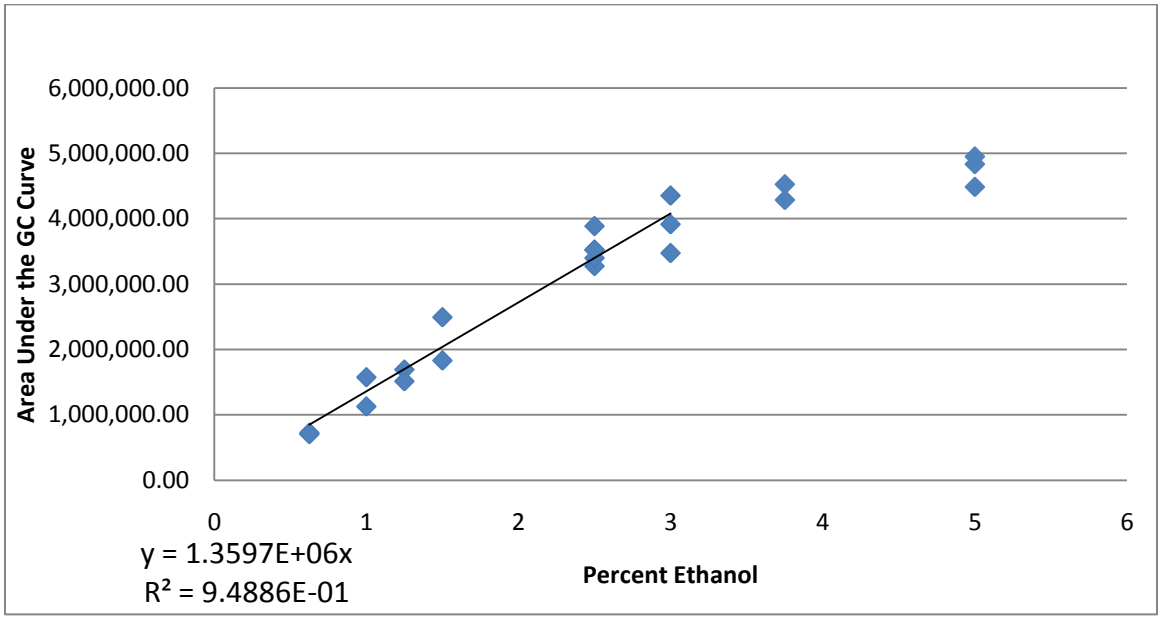


Figure 7: Standard Curve for GC Area to Percent Ethanol

In order to confirm the ethanol peak in the GC spectrograph, the sample from the experiment was run through the GC column, and then spiked with additional ethanol. This was then run through the GC a second time to observe if the peak assumed to be ethanol from the original sample increased. The percent ethanol was plotted against time. When both the ethanol concentration and cell count plots leveled off and no longer increased with time, it was assumed that all substrate had been used and the run was terminated.

## Run 1

Run 1 was conducted to compare all four strains of yeast. Flasks A through D contained identical media with 20 g/L of glucose. Flasks E and F compared the wild yeast to the strain including all genetic modifications in the presence of xylose. A summary can be seen in Table 2.

Table 2: Summary of Run 1

| Flask | Yeast | Glucose (g/L) | Xylose (g/L) |
|---|---|---|---|
| A | Wild type | 20 | 0 |
| B | +LTC | 20 | 0 |
| C | ΔGre3 | 20 | 0 |
| D | +LTC, ΔGre3 | 20 | 0 |
| E | Wild type | 10 | 10 |
| F | +LTC, ΔGre3 | 10 | 10 |

After this run due to time constraints it was determined to only compare the wild type and the strain containing all modifications, as previous work with these strains had shown both genetic modifications are necessary for optimal xylose metabolism.

## Runs 2 and 3

Runs 2 and 3 were designed to determine how much the genetic modifications allowed for the utilization of xylose. Only the wild type and fully recombinant strains were studied. The ratio of glucose to xylose was varied to allow for the direct comparison of the two strains of yeast, as seen in Table 3 below.

Table 3: Summary of Runs 2 and 3

| Flask | Yeast | Glucose (g/L) | Xylose (g/L) |
|---|---|---|---|
| A | Wild type | 10 | 0 |
| B | Wild Type | 6 | 4 |
| C | Wild Type | 4 | 6 |
| D | +LTC, ΔGR3 | 10 | 0 |
| E | +LTC, ΔGR3 | 6 | 4 |
| F | +LTC, ΔGR3 | 4 | 6 |

## Run 4

Run 4 was designed to measure if the modified yeast could utilize xylose when there was no glucose present, as well as to further investigate the required ratio of glucose to xylose to perform better than wild type.

**Table 4: Summary of Run 4**

| Flask | Yeast | Glucose (g/L) | Xylose (g/L) |
|---|---|---|---|
| A | Wild type | 0 | 10 |
| B | Wild Type | 1 | 9 |
| C | Wild Type | 2 | 8 |
| D | +LTC, ΔGR3 | 0 | 10 |
| E | +LTC, ΔGR3 | 1 | 9 |
| F | +LTC, ΔGR3 | 2 | 8 |

# Results and Discussion

Four experiments in total were performed, each comparing wild type *S. cerevisiae* to *S. cerevisiae* with different genetic modifications. As detailed in the background, these modifications are removal of the gre3 gene which can inhibit metabolism into ethanol, addition of nine genes to aid in xylose metabolism, and both of these modifications together. The first experiment concentrated on comparing each genetic modification to each other as well as the wild type. The second, third and fourth experiments then focused on comparison of the wild type to the yeast with both genetic modifications, using different combinations of xylose and glucose.

## Experiment 1

The first experiment performed compared all four yeast strains using all glucose, as well as comparing the wild type to yeast with both genetic modifications in a mixture of xylose and glucose. When the four different strains of yeast were compared under the same substrate conditions, it was clear that the insertion of the LTC cassette greatly impact the growth rate. The deletion of the Gre3 gene has little impact on the growth rate, as seen in Figure 8 below.

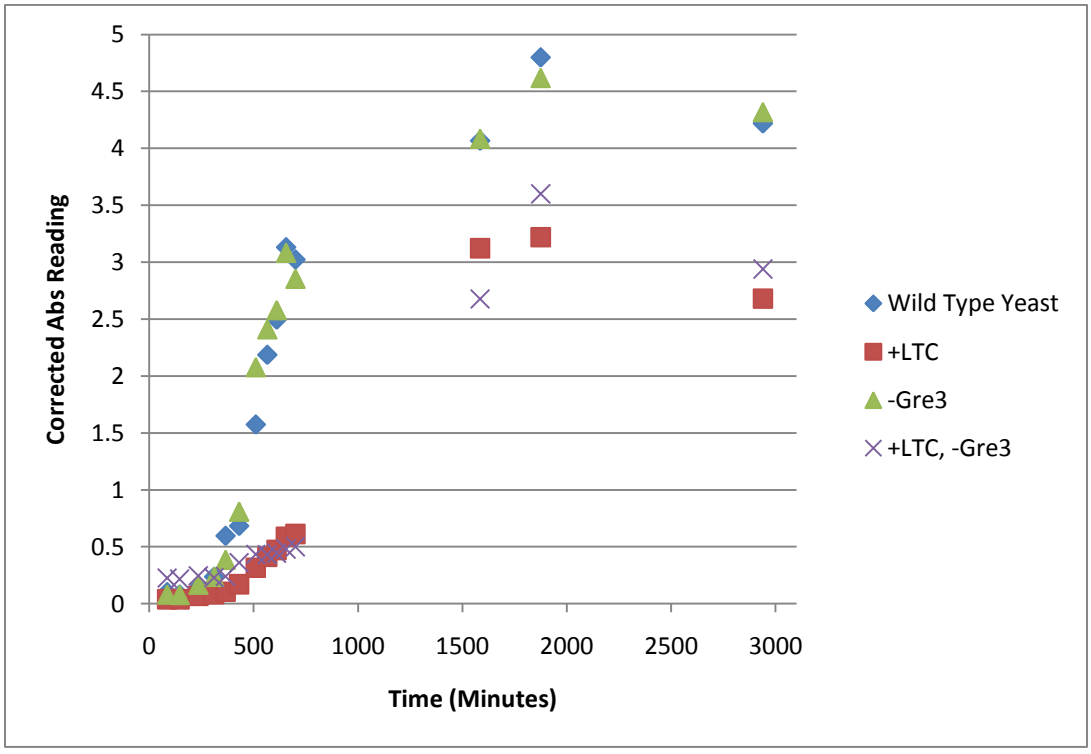


Figure 8: Growth rate for the 4 types of yeast given 20g/L Glucose

In this run it can be noted that the species of yeast containing all genetic modifications has a higher initial cell count. This is due to the fact that the media may have been contaminated prior to

autoclaving, as evidenced by a slightly cloudy solution. In this run insufficient data was collected to accurately report on the ethanol production. At this time, the rate of the reaction was unknown which resulted in a lack of sampling during exponential growth.

Additionally, the wild type yeast and the strain containing all modifications were compared under a 50/50 weight percent of glucose and xylose. The same impact on growth rate as noted above was seen from the LTC additions, shown in Figure 9.

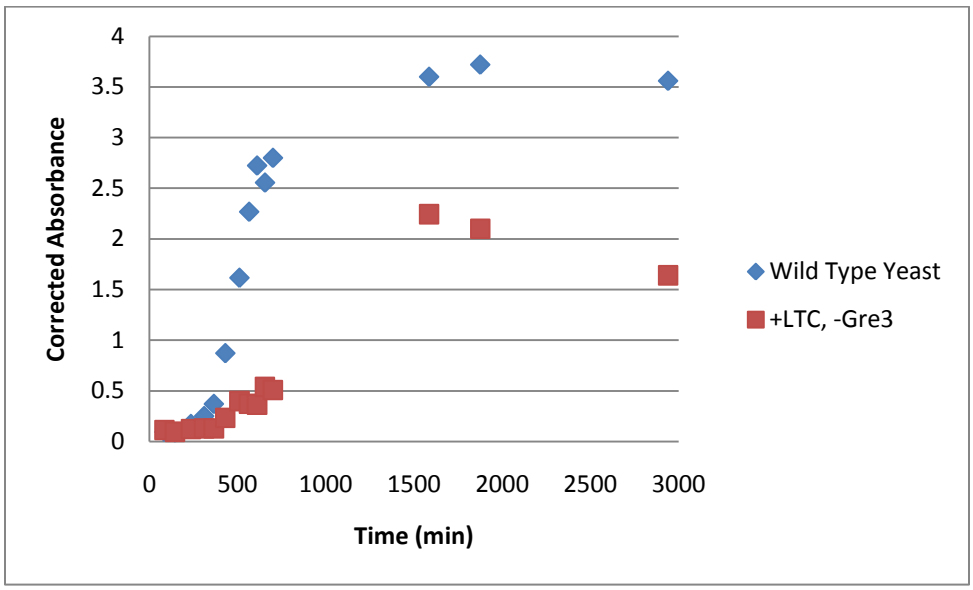


Figure 9: Cell Growth Comparing strains of yeast with 10g/L Glucose and 10g/L Xylose

There was also a lack of data on the ethanol production to draw specific conclusions, however is can be noted in Figure 10 that the maximum ethanol concentration is roughly the same for both strains of yeast. In this run the samples were not run through the GC multiple times to determine an average area, and therefore must be assumed to have a greater margin of error. However, this data when combined with data discussed below suggests that the modified yeast was most likely utilizing the xylose since it does not use glucose to the same degree that the wild type yeast does.

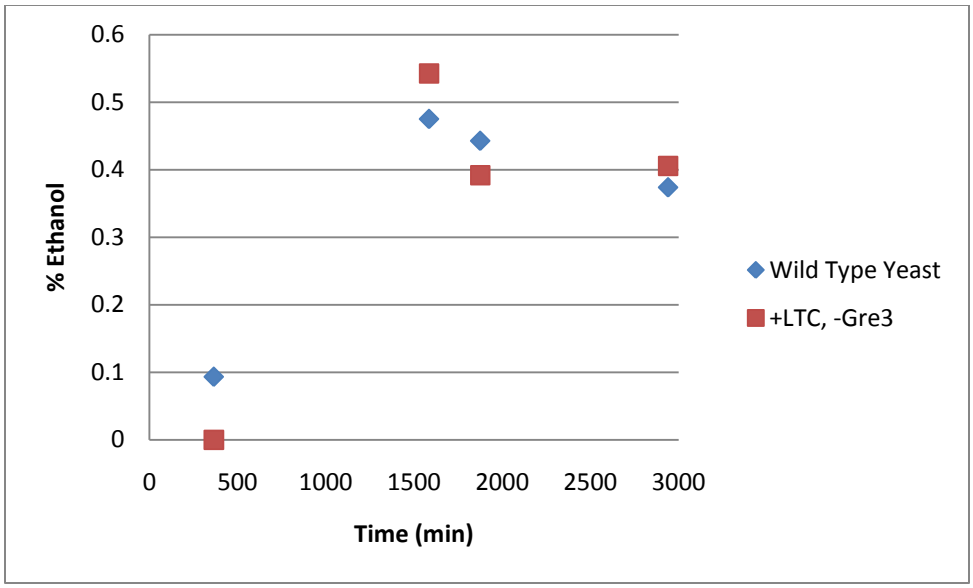


Figure 10: Ethanol Production comparing strains of yeast with 10g/L Glucose and 10g/L Xylose

## Experiments 2 and 3

Experiments 2 and 3 were run identically, comparing the strain of yeast containing both genetic modifications to the wild type yeast under various concentrations of glucose and xylose. The cell growth was monitored using the Spec 20D. When directly compared to the wild yeast, the modified yeast had a delayed exponential growth phase and never reached the same concentration level as the wild yeast. In all cases, the wild yeast began the exponential growth phase at about 400 minutes after being introduced to the media. The modified yeast, however, did not begin this phase until about 600 minutes. In both cases, the yeast began leveling off at about 1000 minutes, suggesting that all substrate had been used. The maximum ethanol concentration is also seen at this time.

### Cell Growth

A seen in Figure 11 below, the wild yeast reached a concentration corresponding to an absorbance reading of 3.5. Typically, the absorbance readings would be correlated to the dry weight of the cells; this was not done in this study due to equipment constraints and because the trend was determined important, not the actual cell count. The modified yeast however reached a maximum at a concentration corresponding to a reading of 2.3, or about two thirds of the concentration of the wild yeast.

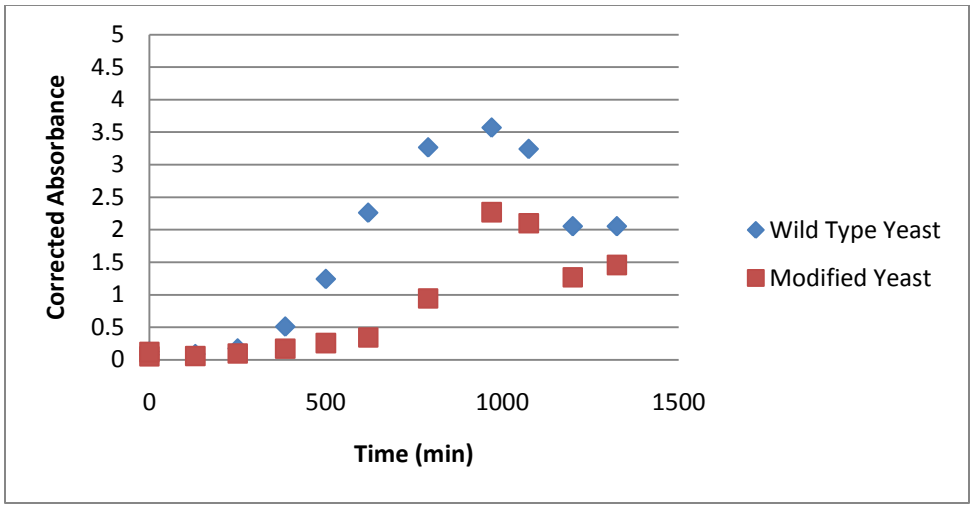


Figure 11: Cell Growth in 10g/L of Glucose

Figure 12 illustrates the same information as above, but using a substrate mixture of 6 g/L of glucose and 4 g/L of xylose. Because the wild yeast cannot utilize the xylose, it was expected that the cell concentration of wild yeast would be lower than when 10 g/L of glucose was used. The modified yeast however could utilize the xylose and the cell concentration was expected to be similar too or only slightly less than the first case. As seen below, the wild yeast cell concentration was actually higher than when only glucose was used for the first run. This is most likely due human error in measuring the substrate concentration. The data was collected over the course of two runs, and performed similarly in the exponential growth phase. The high cell concentration seen between 700 minutes and 1100 minutes was seen in the first run, however lower concentrations seen after 1200 minutes were from the second run. Because of this error, it is difficult to compare the ratios with other substrate concentrations.

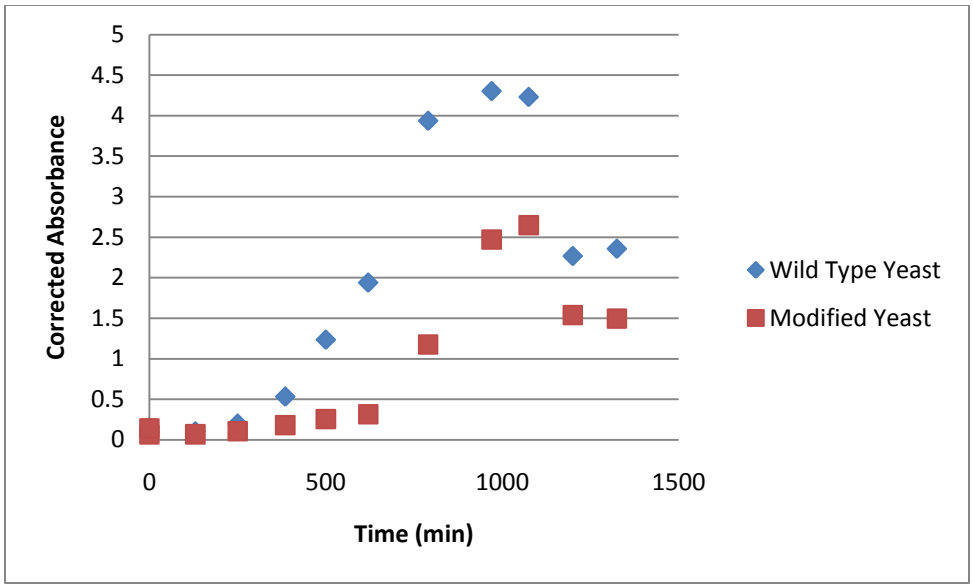


Figure 12: Cell Growth in 6g/L Glucose and 4 g/L Xylose

When 4g/L of glucose in combination with 6g/L of xylose was used, the wild yeast was expected to have a cell count much lower than when only glucose was used. Again, it was expected that the modified yeast would utilize the xylose and be similar to the all glucose case. The wild yeast reached an absorbance reading of 3.3, and the recombinant yeast reached 2.4, or nearly 75% of the concentration of the wild yeast, seen Figure 13. As expected this is higher than when only glucose was used.

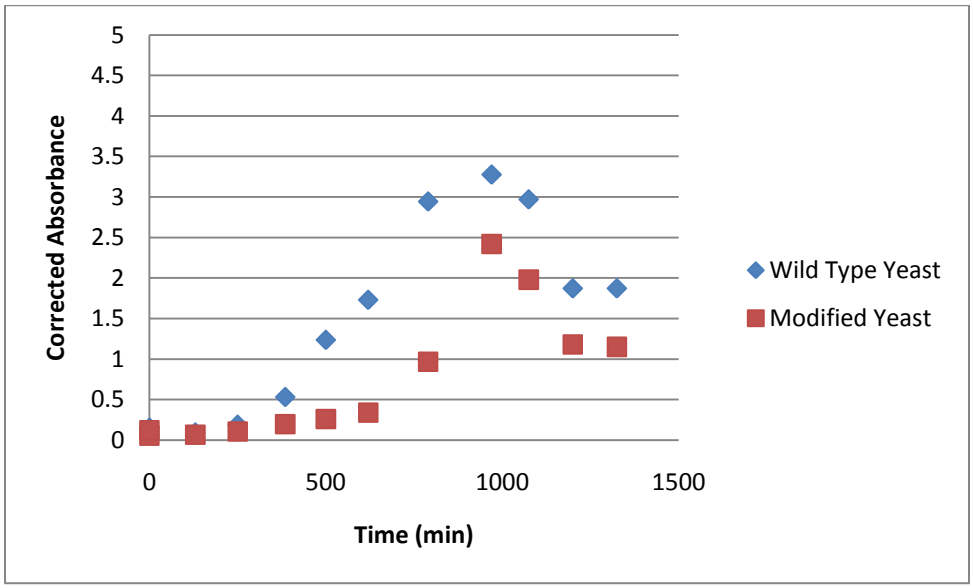


Figure 13: Cell Growth in 4 g/L Glucose and 6g/L Xylose

The growth rate of the yeast directly correlates to the rate of ethanol production. In order to calculate the growth rate of the yeast, the previous plots of cell growth versus time, see Figure 11

through Figure 13, were graphed on a semi log scale. The exponential growth phase appears linear on this scale, making it easy to determine the actual time where the exponential growth occurred. A trend line was plotted for the linear time period. The slope of the trend line corresponds to the general differential growth rate equation, $\frac{dx}{dt} = rx$, see Figure 14 below as an example.

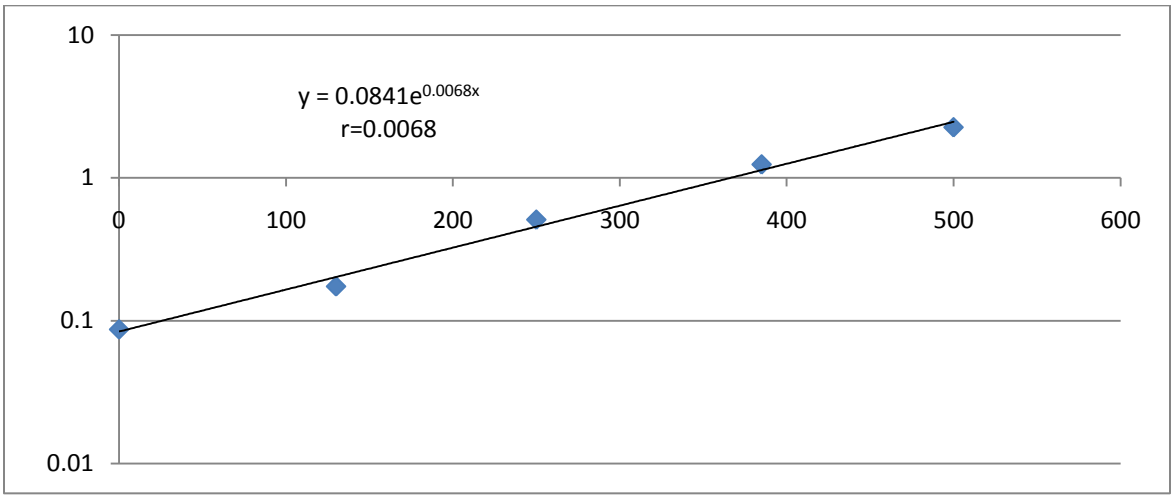


Figure 14: Reaction rate for Wild Yeast with all Glucose

Table 5 summarizes and compares the growth rate for the two strains of yeast. The growth rate 'r' is 0.006 $min^{-1}$ for the wild yeast under all three substrate scenarios. This is because the rate is a function of the organism and not the substrate. The reaction rate for the modified yeast was only 0.003 $min^{-1}$, or half the rate of the wild type yeast. This could to lead to issues when scaling up to an industrial production level. The slower growth rate leads to a slower ethanol production rate, hindering the industrial supply. Figure B1 through Figure B6 in Appendix B illustrate how these values were determined.

Table 5: Growth Rate Comparison

| Yeast | Substrate (g glucose/ g xylose) | Growth Rate r ($min^{-1}$) |
|---|---|---|
| Wild | 10/0 | 0.0068 |
| Wild | 6/4 | 0.0061 |
| Wild | 4/6 | 0.0062 |
| Modified | 10/0 | 0.0036 |
| Modified | 6/4 | 0.0031 |
| Modified | 4/6 | 0.0033 |

## Ethanol Production

Next, the actual amount of ethanol produced in each case was compared. 10.00 g/L of glucose corresponds to 0.0555 molarity of glucose, as seen in Equation 1.

**Equation 1: Moles of Glucose**

$$\frac{10.00 \pm .005 g\ Glucose}{L\ Media} \times \frac{1\ mol\ Glucose}{180.16\ g\ Glucose} = \frac{0.0555 \pm 0.00006\ mol\ Glucose}{L\ Media}$$

The glucose fermentation reaction $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ shows that 2 moles of ethanol are produced from every mole of glucose. This means that at most, 0.111 moles of ethanol, or 0.6% ethanol by volume can be produced. It is not expected that this much ethanol will be produced as some of it is used for cell production and some is converted to carbon dioxide and other compounds.

**Equation 2: Ethanol Produced**

$$\frac{0.111\ mol\ Etoh}{L\ Media} \times \frac{46.07\ g\ Etoh}{1\ mol\ Etoh} \times \frac{L\ Etoh}{789\ g\ Etoh} = \frac{0.00648\ L\ Etoh}{L\ Media} = 0.6\%\ volume\ Etoh$$

As seen in Figure 15, the wild yeast produced 0.5% ethanol by volume, as expected. The modified yeast produced about 0.34% ethanol by volume. It can be expected that since the metabolism pathway of the recombinant yeast has been modified, it may not be as effective as the wild type yeast at fermenting glucose.

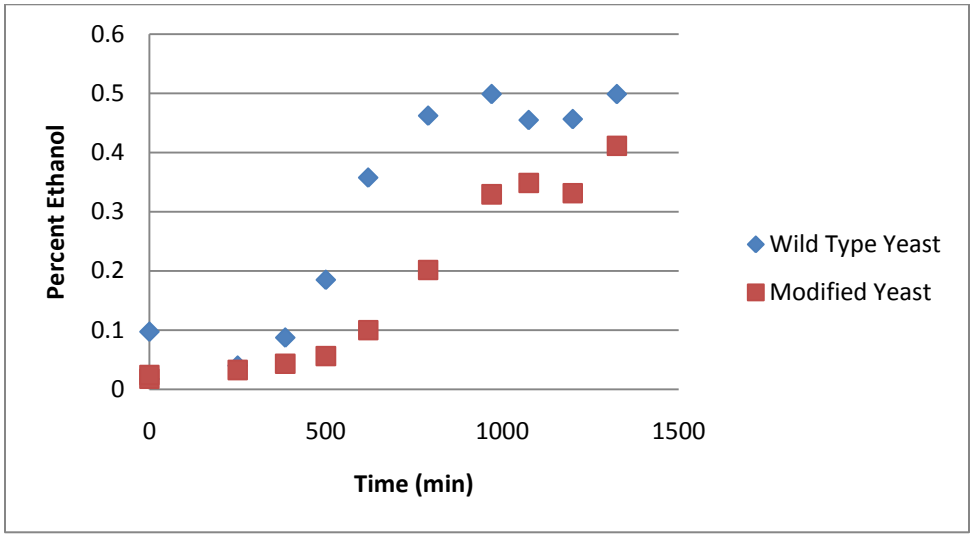


**Figure 15: Ethanol Production using all Glucose**

The ethanol yield was then determined. The wild typed yeast produced 0.395 grams of ethanol per gram of substrate, as seen in Equation 3 below. The modified yeast however only had a yield of 0.268 g ethanol per gram of substrate, as seen in Equation 4.

**Equation 3: Ethanol Yield for Wild Type Yeast and 10 g/L glucose**

$$\frac{.005\ L\ Etoh}{L\ Media} \times \frac{1\ L\ Media}{10\ g\ Glucose} \times \frac{789\ g\ Etoh}{1\ L\ Etoh} = \frac{0.395\ g\ Etoh}{g\ Glucose}$$

**Equation 4: Ethanol Yield for Modified Yeast and 10 g/L glucose**

$$\frac{.0034\ L\ Etoh}{L\ Media} \times \frac{1\ L\ Media}{10\ g\ Glucose} \times \frac{789\ g\ Etoh}{1\ L\ Etoh} = \frac{0.268\ g\ Etoh}{g\ Glucose}$$

For the second case there was 0.0333 moles of glucose available and 0.0266 moles of xylose, as calculated in Equation 5 and Equation 6.

**Equation 5: Glucose in Media**

$$\frac{6.00 \pm .005\ g\ Glucose}{L\ Media} \times \frac{1\ mol\ Glucose}{180.16\ g\ Glucose} = \frac{0.0333 \pm .00006\ mol\ Glucose}{L\ Media}$$

**Equation 6: Xylose in Media**

$$\frac{4.00 \pm .005\ g\ Xylose}{L\ Media} \times \frac{1\ mol\ Xylose}{150.13\ g\ Xylose} = \frac{0.0266 \pm .00003\ mol\ Xylose}{L\ Media}$$

The xylose fermentation reaction $3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2$ shows that there is a 3:5 mole ratio of xylose to ethanol. This means that 0.0666 moles of ethanol can potentially be produced from the glucose and 0.0443 moles of ethanol formed from the xylose. This means that at most 0.39% volume of ethanol can be expected from the wild yeast and 0.65% volume ethanol can be expected from the modified yeast, as seen below.

**Equation 7: Ethanol produced by Wild Yeast**

$$\frac{0.0666\ mol\ Etoh}{L\ Media} \times \frac{46.07\ g\ Etoh}{1\ mol\ Etoh} \times \frac{L\ Etoh}{789\ g\ Etoh} = \frac{0.00389\ L\ Etoh}{L\ Media} = 0.39\%\ volume\ Etoh$$

**Equation 8: Ethanol produced by Modified Yeast**

$$\frac{(0.0666 + .0443)\ mol\ Etoh}{L\ Media} \times \frac{46.07\ g\ Etoh}{1\ mol\ Etoh} \times \frac{L\ Etoh}{789\ g\ Etoh} = \frac{0.00648\ L\ Etoh}{L\ Media} = 0.65\%\ volume\ Etoh$$

These determinations rely on the assumption that wild yeast does not utilize any xylose, and the modified yeast consumes all of the glucose and xylose. As seen in Figure 16 below, the modified yeast produces about 0.51% ethanol by volume. This value is very reasonable, as the recombinant yeast does

not fully utilize the glucose to the extent that the wild yeast did, shown in Experiment 1. The modified yeast produced about 78.5% of the maximum amount of ethanol. The modified yeast also produced more ethanol then would be possible if only the glucose was used, confirming that the yeast is utilizing the xylose.

The wild type yeast produced ethanol in excess of the expected amount. However, as discussed previously, there was most likely a human error in substrate measurements. When taken into consideration with the higher than expected cell growth, the high concentration of ethanol is reasonable. The lower ethanol concentration value seen in Figure 16 at 1100 minutes is under 4% and should be taken as the maximum. This value is from Experiment 3, where no significant errors were expected. Higher values after 800 minutes are from Experiment 2, most likely from previously discussed errors.

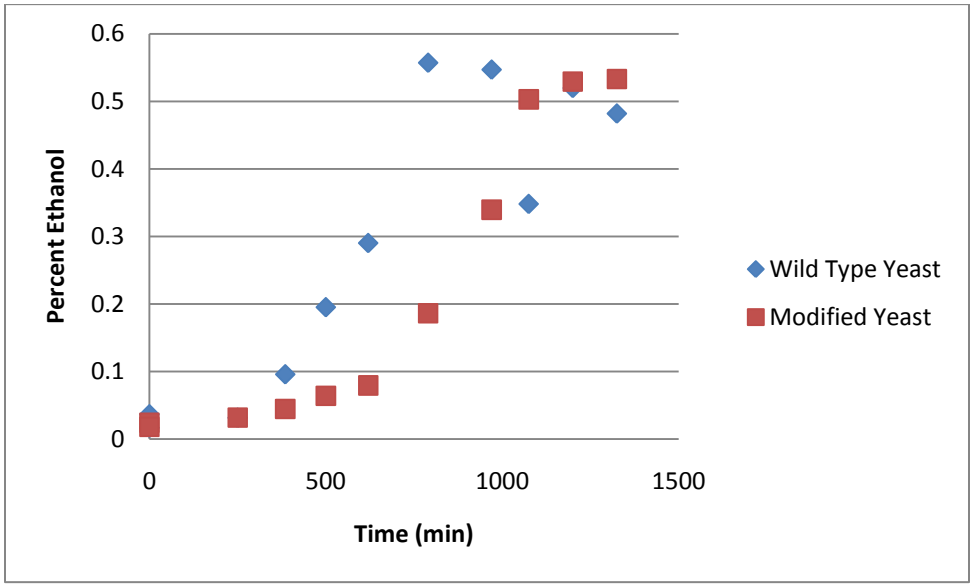


Figure 16: Ethanol Production using 6g/L glucose and 4g/L xylose

The ethanol yield was again determined. The total amount of substrate used was again 10 grams, however in this case it was a combination of glucose and xylose. The wild yeast only utilized the glucose, and therefore has a much lower yield then the modified yeast as seen in Equation 9 and Equation 10 below.

**Equation 9: Ethanol Yield for Wild Type Yeast and 6 g/L glucose, 4 g/L xylose**

$$\frac{.0035\ L\ Etoh}{L\ Media} \times \frac{1\ L\ Media}{10\ g\ Substrate} \times \frac{789\ g\ Etoh}{1\ L\ Etoh} = \frac{0.276\ g\ Etoh}{g\ Glucose}$$

**Equation 10: Ethanol Yield for Modified Yeast and 6 g/L glucose, 4 g/L xylose**

$$\frac{.0051\ L\ Etoh}{L\ Media} \times \frac{1\ L\ Media}{10\ g\ Substrate} \times \frac{789\ g\ Etoh}{1\ L\ Etoh} = \frac{0.402\ g\ Etoh}{g\ Glucose}$$

For the third case, 4 g/L glucose and 6 g/L xylose, there are 0.0222 moles of glucose and 0.0340 moles of xylose available, shown below.

**Equation 11: Glucose in Media**

$$\frac{4.00 \pm .005\ g\ Glucose}{L\ Media} \times \frac{1\ mol\ Glucose}{180.16\ g\ Glucose} = \frac{0.0222 \pm .00006\ mol\ Glucose}{L\ Media}$$

**Equation 12: Xylose in Media**

$$\frac{6.00 \pm .005\ g\ Xylose}{L\ Media} \times \frac{1\ mol\ Xylose}{150.13\ g\ Xylose} = \frac{0.0340 \pm .00003\ mol\ Xylose}{L\ Media}$$

This means that 0.0444 moles of ethanol can potentially be produced from glucose and 0.0567 moles of ethanol from xylose. At most 0.26% volume of ethanol can be expected from the wild yeast and 0.59% volume ethanol can be expected from the modified yeast when using the same assumptions regarding sugar metabolism previously stated. This is shown in Equation 13 and Equation 14 below.

**Equation 13: Ethanol produced by Wild Yeast**

$$\frac{0.0444\ mol\ Etoh}{L\ Media} \times \frac{46.07\ g\ Etoh}{1\ mol\ Etoh} \times \frac{L\ Etoh}{789\ g\ Etoh} = \frac{0.00259\ L\ Etoh}{L\ Media} = 0.26\%\ volume\ Etoh$$

**Equation 14: Ethanol produced by Modified Yeast**

$$\frac{(0.0444 + .0567)\ mol\ Etoh}{L\ Media} \times \frac{46.07\ g\ Etoh}{1\ mol\ Etoh} \times \frac{L\ Etoh}{789\ g\ Etoh} = \frac{0.00590\ L\ Etoh}{L\ Media} = 0.59\%\ volume\ Etoh$$

As seen in Figure 17, the wild yeast produced around 0.3% volume ethanol. While this is higher than expected it is close to the expected value and can be assumed to be within the margin of error. The modified yeast however only produced 0.35% by volume of ethanol. This is only 59.3% of the maximum amount as compared to the 78.5% seen in the previous case. This would suggest that this strain of yeast utilizes the xylose even less efficiently then it utilizes the glucose.

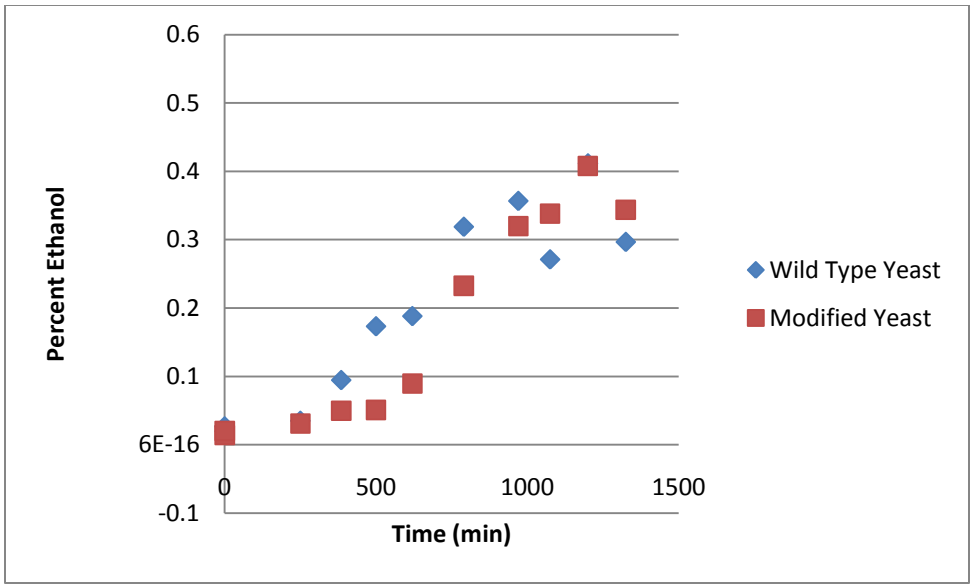


Figure 17: Ethanol Production using 4 g/L glucose and 6 g/L xylose

The ethanol yield was much lower for this substrate ratio. The wild yeast again only utilized the glucose, and therefore a much lower yield was expected. The modified yeast should have utilized both the glucose and xylose and should have maintained a higher ethanol yield, but it does not seem to use the xylose to the same degree with the smaller amount of glucose available. See Equation 15and Equation 16below.

Equation 15: Ethanol Yield for Wild Type Yeast and 4 g/L glucose, 6 g/L xylose

$$\frac{.003\ L\ Etoh}{L\ Media} \times \frac{1\ L\ Media}{10\ g\ Substrate} \times \frac{789\ g\ Etoh}{1\ L\ Etoh} = \frac{0.237\ g\ Etoh}{g\ Glucose}$$

Equation 16: Ethanol Yield for Modified Yeast and 4 g/L glucose, 6 g/L xylose

$$\frac{.0035\ L\ Etoh}{L\ Media} \times \frac{1\ L\ Media}{10\ g\ Substrate} \times \frac{789\ g\ Etoh}{1\ L\ Etoh} = \frac{0.276\ g\ Etoh}{g\ Glucose}$$

The higher than expected values for the wild type yeast may also suggest that it is utilizing the xylose to some small degree. Wild type yeast may have the ability to utilize xylose for cell growth, leaving additional glucose for ethanol fermentation. Further studies would have to be conducted to confirm this theory. Also, there is a small amount of ethanol at time zero, but this amount is not determinable within the margin of error and is therefore assumed to be zero. This ethanol is generated in the inoculation tubes prior to the beginning of the experiment.

## Experiment 4

Previous research with this strain of recombinant yeast suggested that the yeast could not grow and thrive on xylose alone, and glucose was needed to 'jumpstart' the reaction. The yeast were grown in a solution containing 10g/L of substrate, with only a limited amount of glucose, ranging from 0g/L to 2 g/L. This study confirmed that glucose is necessary, and approximated how much glucose was needed to start the reaction.

### Cell Growth

Overall there was limited cell growth with both strains of yeast, even when 2 g/L of glucose was used. As expected, the wild yeast did not grow without any glucose. When given 10g/L glucose the wild type yeast grew to a concentration corresponding to an absorbance reading of 3.5, much higher than growth seen in Figure 18 below.

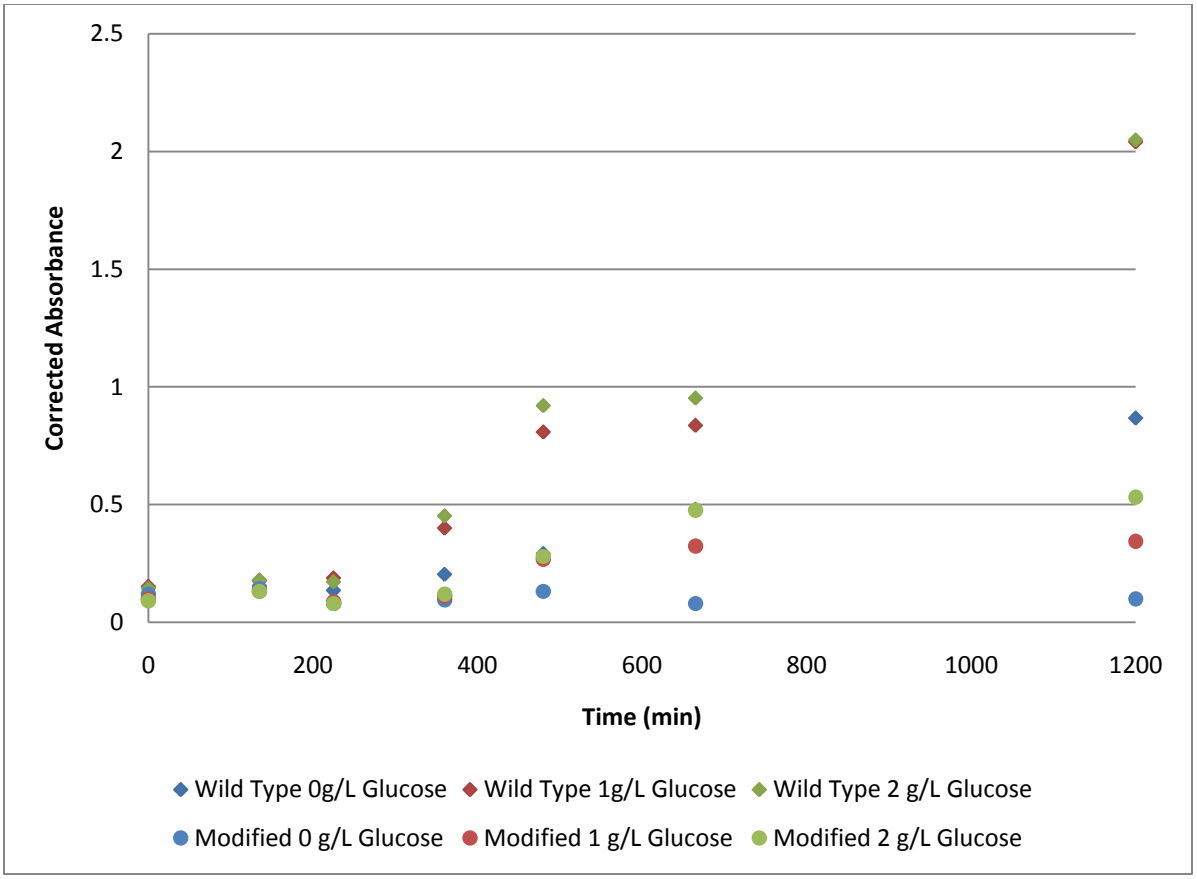


Figure 18: Cell Growth under limited glucose conditions

### Ethanol Production

The ethanol production was analyzed in the same fashion as in Experiments 2 and 3. When no glucose was used, no discernable amount of ethanol was produced by either the wild type or the

modified yeast, seen in Figure 19. Therefore it can be assumed that the modified yeast does need some glucose to start the fermentation reaction. Also, as expected the wild type yeast did not produce any ethanol since it does not have the ability to metabolize xylose into ethanol.

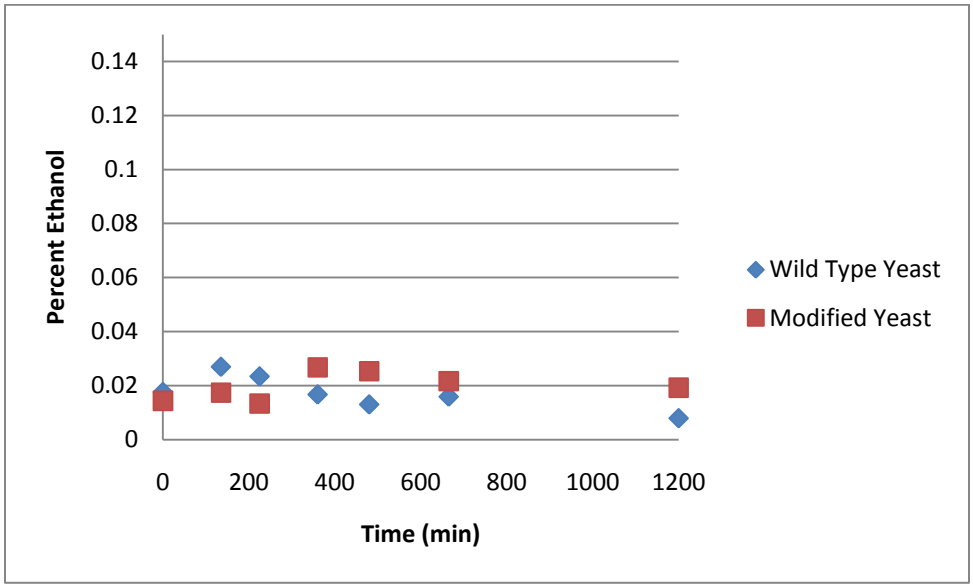


Figure 19: Ethanol Production with no Glucose

For the second case, where 1 g/L of glucose was used, 0.0056 moles of glucose was available for fermentation and could potentially produce at most 0.0112 moles of ethanol. For the wild type yeast this means that at most 0.06% by volume of ethanol was expected. There is however an additional 0.0599 moles of xylose available to the modified yeast, which could produce at most 0.100 additional moles of ethanol. If the modified yeast used the xylose the expected ethanol concentration would be 0.65% by volume, shown below.

Equation 17: Glucose in Media

$$\frac{1.00 \pm .005\ g\ Glucose}{L\ Media} \times \frac{1\ mol\ Glucose}{180.16\ g\ Glucose} = \frac{0.0056 \pm .00006\ mol\ Glucose}{L\ Media}$$

Equation 18: Xylose in Media

$$\frac{9.00 \pm .005\ g\ Xylose}{L\ Media} \times \frac{1\ mol\ Xylose}{150.13\ g\ Xylose} = \frac{0.0599 \pm .00003\ mol\ Xylose}{L\ Media}$$

Equation 19: Ethanol produced by Wild Yeast

$$\frac{0.0112\ mol\ Etoh}{L\ Media} \times \frac{46.07\ g\ Etoh}{1\ mol\ Etoh} \times \frac{L\ Etoh}{789\ g\ Etoh} = \frac{0.00065\ L\ Etoh}{L\ Media} = 0.06\%\ volume\ Etoh$$

Equation 20: Ethanol produced by Modified Yeast

$$\frac{(0.0112 + .100)\ mol\ Etoh}{L\ Media} \times \frac{46.07\ g\ Etoh}{1\ mol\ Etoh} \times \frac{L\ Etoh}{789\ g\ Etoh} = \frac{0.00649\ L\ Etoh}{L\ Media} = 0.65\%\ volume\ Etoh$$

As seen in Figure 20 however, the modified yeast performed nearly the same as the wild type yeast, producing 0.06% ethanol by volume. This suggests that both types of yeast use the glucose and were not able to utilize the xylose.

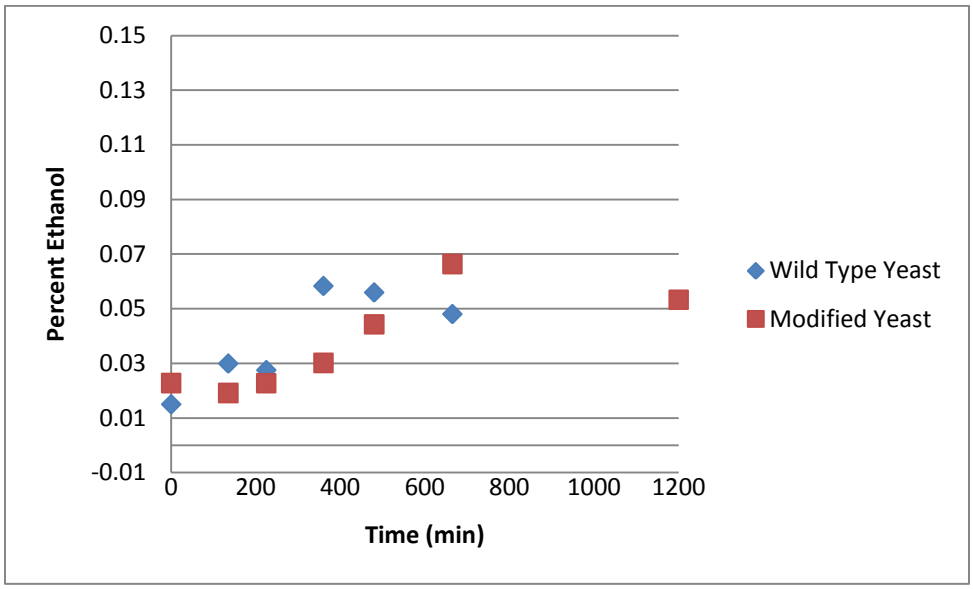


Figure 20: Ethanol Production with 1 g/L Glucose and 9g/L Xylose

The same analysis for 2 g/L of glucose concluded that 0.0111 moles of glucose can produce 0.0222 moles of ethanol, and 0.0533 moles of xylose can produce 0.0888 moles of ethanol. This would allow for the wild type yeast to produce 0.13% ethanol by volume and the modified yeast to produce 0.65% ethanol by volume, shown below.

Equation 21: Glucose in Media

$$\frac{2.00 \pm .005\ g\ Glucose}{L\ Media} \times \frac{1\ mol\ Glucose}{180.16\ g\ Glucose} = \frac{0.0111 \pm .00006\ mol\ Glucose}{L\ Media}$$

Equation 22: Xylose in Media

$$\frac{8.00 \pm .005\ g\ Xylose}{L\ Media} \times \frac{1\ mol\ Xylose}{150.13\ g\ Xylose} = \frac{0.0533 \pm .00003\ mol\ Xylose}{L\ Media}$$

Equation 23: Ethanol produced by Wild Yeast

$$\frac{0.0222\ mol\ Etoh}{L\ Media} \times \frac{46.07\ g\ Etoh}{1\ mol\ Etoh} \times \frac{L\ Etoh}{789\ g\ Etoh} = \frac{0.00130\ L\ Etoh}{L\ Media} = 0.13\%\ volume\ Etoh$$

Equation 24: Ethanol produced by Modified Yeast

$$\frac{(0.0222 + .0888) mol\ Etoh}{L\ Media} \times \frac{46.07\ g\ Etoh}{1\ mol\ Etoh} \times \frac{L\ Etoh}{789\ g\ Etoh} = \frac{0.00648\ L\ Etoh}{L\ Media} = 0.65\%\ volume\ Etoh$$

However, as seen in Figure 21, the same situation occurs where the modified yeast performs similarly to the wild type yeast. Both types produce about 0.1% ethanol by volume.

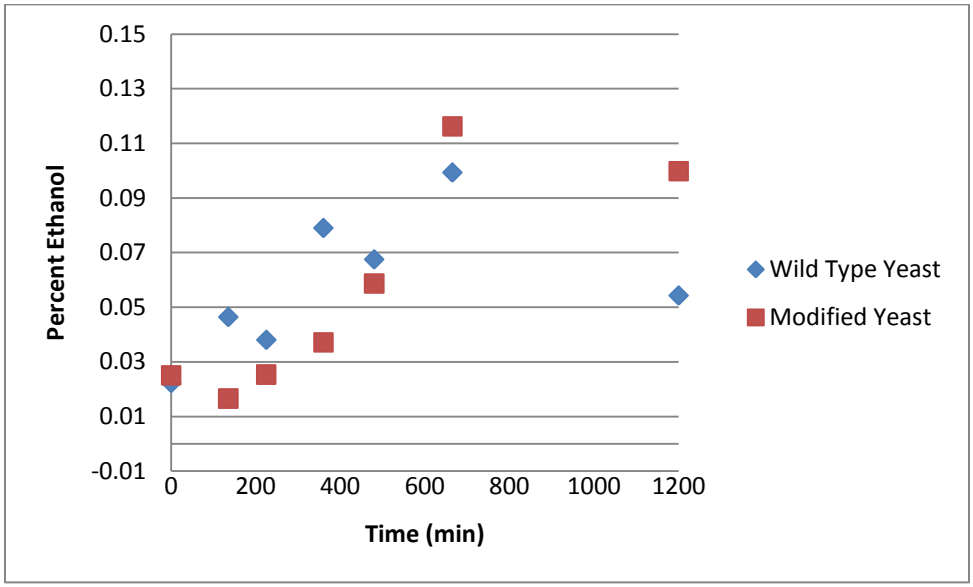


Figure 21: Ethanol Production with 2 g/L Glucose and 8 g/L Xylose

## Conclusions and Recommendations

When examining the results of the modified yeast's performance at the various substrate concentrations, it is clear that the yeast utilizes xylose if an adequate amount of glucose is also supplied. Figure 22 below suggests that the optimum ratio of glucose to xylose is around 6:4 by mass. When 20% of glucose or less is supplied, the modified yeast performs the same as the wild type yeast, suggesting that it is only using the glucose. When 4 g/L of glucose is supplied with 6 g/L of xylose, the modified yeast performs nearly the same as when 10g/L of glucose is supplied. This suggests that in order to maximize performance, at least half of the substrate supplied should be glucose.

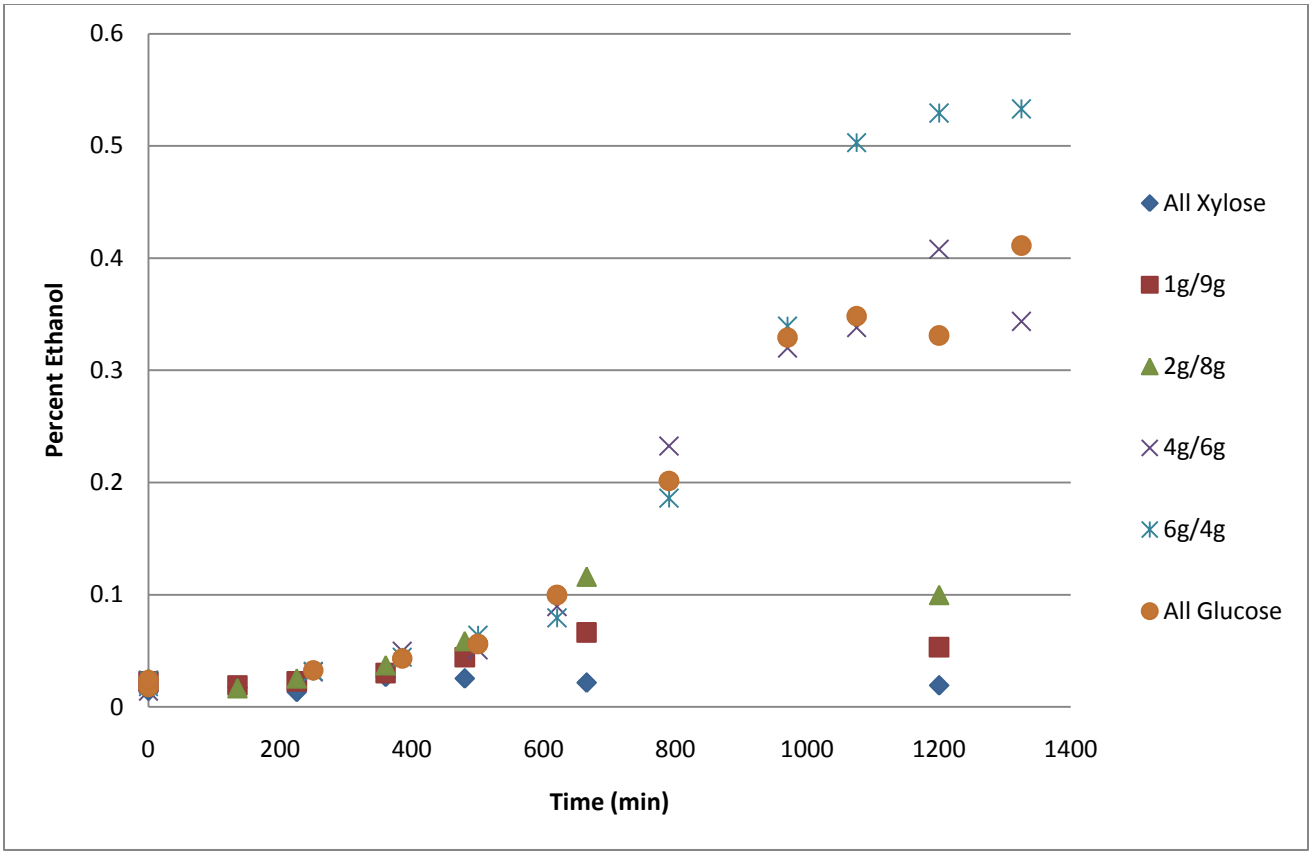


Figure 22: Comparing the Modified Yeast's performance at various substrate concentrations

The slower growth rate of the modified yeast is not deemed to be an issue; however it is an important consideration when scaling up the process to an industry level. Altering reactor conditions, such as temperature, pH, mixing speed and other variables may reduce the lag time and increase the growth rate. Because of this, further studies should be conducted.

Additionally, it is important to bear in mind that fermentation is only one part of the complex process of turning raw biomass into ethanol. The ability to ferment xylose is only important if the

xylose can be obtained from the raw materials. Better hydrolysis methods are required for the process to become sustainable and further research in this area is required.

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# Appendix A: Raw Data Collected

Table A1: Experiment 1 Raw Data

| Time | Flask | Dilution | Abs | Corrected Abs | Area |
|---|---|---|---|---|---|
| 85 | A | 3 | 0.034 | 0.102 | |
| 85 | B | 3 | 0.013 | 0.039 | |
| 85 | C | 3 | 0.027 | 0.081 | |
| 85 | D | 3 | 0.075 | 0.225 | |
| 85 | E | 3 | 0.032 | 0.096 | |
| 85 | F | 3 | 0.038 | 0.114 | |
| 145 | A | 3 | 0.027 | 0.081 | |
| 145 | B | 3 | 0.013 | 0.039 | |
| 145 | C | 3 | 0.026 | 0.078 | |
| 145 | D | 3 | 0.072 | 0.216 | |
| 145 | E | 3 | 0.03 | 0.09 | |
| 145 | F | 3 | 0.032 | 0.096 | |
| 235 | A | 3 | 0.048 | 0.144 | |
| 235 | B | 3 | 0.023 | 0.069 | |
| 235 | C | 3 | 0.056 | 0.168 | |
| 235 | D | 3 | 0.082 | 0.246 | |
| 235 | E | 3 | 0.058 | 0.174 | |
| 235 | F | 3 | 0.041 | 0.123 | |
| 310 | A | 3 | 0.079 | 0.237 | |
| 310 | B | 3 | 0.028 | 0.084 | |
| 310 | C | 3 | 0.079 | 0.237 | |
| 310 | D | 3 | 0.076 | 0.228 | |
| 310 | E | 3 | 0.084 | 0.252 | |
| 310 | F | 3 | 0.043 | 0.129 | |
| 365 | A | 3 | 0.199 | 0.597 | 2.0500E+05 |
| 365 | B | 3 | 0.035 | 0.105 | 5.7480E+05 |
| 365 | C | 3 | 0.129 | 0.387 | 1.5636E+05 |
| 365 | D | 3 | 0.08 | 0.24 | 5.1088E+04 |
| 365 | E | 3 | 0.124 | 0.372 | 1.2698E+05 |
| 365 | F | 3 | 0.043 | 0.129 | 8.5823E+00 |
| 430 | A | 3 | 0.228 | 0.684 | |
| 430 | B | 3 | 0.057 | 0.171 | |
| 430 | C | 3 | 0.27 | 0.81 | |
| 430 | D | 3 | 0.12 | 0.36 | |
| 430 | E | 3 | 0.291 | 0.873 | |

| | | | | | |
|---|---|---|---|---|---|
| 430 | F | 3 | 0.078 | 0.234 | |
| 510 | A | 7 | 0.225 | 1.575 | |
| 510 | B | 7 | 0.045 | 0.315 | |
| 510 | C | 7 | 0.297 | 2.079 | |
| 510 | D | 7 | 0.062 | 0.434 | |
| 510 | E | 7 | 0.231 | 1.617 | |
| 510 | F | 7 | 0.057 | 0.399 | |
| 565 | A | 9 | 0.243 | 2.187 | |
| 565 | B | 4 | 0.103 | 0.412 | |
| 565 | C | 9 | 0.268 | 2.412 | |
| 565 | D | 4 | 0.108 | 0.432 | |
| 565 | E | 9 | 0.252 | 2.268 | |
| 565 | F | 4 | 0.093 | 0.372 | |
| 610 | A | 12 | 0.208 | 2.496 | |
| 610 | B | 4 | 0.118 | 0.472 | |
| 610 | C | 12 | 0.215 | 2.58 | |
| 610 | D | 4 | 0.111 | 0.444 | |
| 610 | E | 12 | 0.227 | 2.724 | |
| 610 | F | 4 | 0.091 | 0.364 | |
| 655 | A | 12 | 0.261 | 3.132 | |
| 655 | B | 4 | 0.147 | 0.588 | |
| 655 | C | 12 | 0.257 | 3.084 | |
| 655 | D | 4 | 0.12 | 0.48 | |
| 655 | E | 12 | 0.213 | 2.556 | |
| 655 | F | 4 | 0.135 | 0.54 | |
| 700 | A | 14 | 0.216 | 3.024 | |
| 700 | B | 4 | 0.153 | 0.612 | |
| 700 | C | 14 | 0.204 | 2.856 | |
| 700 | D | 4 | 0.125 | 0.5 | |
| 700 | E | 14 | 0.2 | 2.8 | |
| 700 | F | 4 | 0.127 | 0.508 | |
| 1585 | A | 18 | 0.226 | 4.068 | 7.8660E+05 |
| 1585 | B | 14 | 0.223 | 3.122 | 1.0835E+06 |
| 1585 | C | 18 | 0.227 | 4.086 | 1.5778E+06 |
| 1585 | D | 12 | 0.223 | 2.676 | 1.0081E+06 |
| 1585 | E | 18 | 0.2 | 3.6 | 6.4609E+05 |
| 1585 | F | 12 | 0.187 | 2.244 | 7.3753E+05 |
| 1875 | A | 20 | 0.24 | 4.8 | 1.3566E+06 |
| 1875 | B | 20 | 0.161 | 3.22 | 1.2360E+06 |
| 1875 | C | 20 | 0.231 | 4.62 | 1.3146E+06 |
| 1875 | D | 20 | 0.18 | 3.6 | 1.3628E+06 |

| | | | | | |
|---|---|---|---|---|---|
| 1875 | E | 20 | 0.186 | 3.72 | 6.0207E+05 |
| 1875 | F | 20 | 0.105 | 2.1 | 5.3267E+05 |
| 2940 | A | 20 | 0.211 | 4.22 | 6.7641E+05 |
| 2940 | B | 20 | 0.134 | 2.68 | 1.0627E+06 |
| 2940 | C | 20 | 0.216 | 4.32 | 1.2559E+06 |
| 2940 | D | 20 | 0.147 | 2.94 | 1.7533E+06 |
| 2940 | E | 20 | 0.178 | 3.56 | 5.0837E+05 |
| 2940 | F | 20 | 0.082 | 1.64 | 5.5121E+05 |
| 3205 | A | | | | 1.1354E+06 |
| 3205 | B | | | | 1.1199E+06 |
| 3205 | C | | | | 1.1508E+06 |
| 3205 | D | | | | 1.2996E+06 |
| 3205 | E | | | | 5.18E+05 |
| 3205 | F | | | | 5.19E+05 |
| 5095 | A | | | | 9.44E+05 |
| 5095 | B | | | | 1.04E+06 |
| 5095 | C | | | | 1.27E+06 |
| 5095 | D | | | | 1.20E+05 |
| 5095 | E | | | | 3.14E+05 |
| 5095 | F | | | | 8.04E+05 |

Table A2: Experiment 2 Raw Data

| Time | Flask | Dilution | Abs | Corrected Abs | Area 1 | Area 2 | Avg Area |
|---|---|---|---|---|---|---|---|
| 0 | A | 3 | 0.042 | 0.126 | 1.1999E+05 | 1.4458E+05 | 1.3229E+05 |
| 790 | A | 12 | 0.272 | 3.264 | 6.2311E+05 | 6.3292E+05 | 6.2802E+05 |
| 970 | A | 16 | 0.223 | 3.568 | 6.7863E+05 | 6.7673E+05 | 6.7768E+05 |
| 1075 | A | 18 | 0.18 | 3.24 | 5.3250E+05 | 7.0358E+05 | 6.1804E+05 |
| 1200 | A | 18 | 0.114 | 2.052 | 5.3197E+05 | 7.0844E+05 | 6.2021E+05 |
| 1325 | A | 18 | 0.114 | 2.052 | 8.0104E+05 | 5.5408E+05 | 6.7756E+05 |
| 2520 | A | 18 | 0.129 | 2.322 | 4.0674E+05 | 4.9845E+05 | 4.5259E+05 |
| 0 | B | 3 | 0.05 | 0.15 | 3.8662E+04 | 6.0476E+04 | 4.9569E+04 |
| 790 | B | 16 | 0.246 | 3.936 | 7.1735E+05 | 7.9761E+05 | 7.5748E+05 |
| 970 | B | 18 | 0.239 | 4.302 | 7.6462E+05 | 7.2260E+05 | 7.4361E+05 |
| 1075 | B | 18 | 0.235 | 4.23 | 4.4842E+05 | 4.9793E+05 | 4.7317E+05 |
| 1200 | B | 18 | 0.126 | 2.268 | 6.6550E+05 | 7.4797E+05 | 7.0674E+05 |
| 1325 | B | 18 | 0.131 | 2.358 | 6.4227E+05 | 6.6811E+05 | 6.5519E+05 |
| 2520 | B | 18 | 0.169 | 3.042 | 5.4169E+05 | 5.6031E+05 | 5.5100E+05 |
| 0 | C | 3 | 0.051 | 0.153 | 1.7731E+04 | 5.5637E+04 | 3.6684E+04 |
| 790 | C | 16 | 0.184 | 2.944 | 4.8391E+05 | 3.8365E+05 | 4.3378E+05 |
| 970 | C | 18 | 0.182 | 3.276 | 4.7773E+05 | 4.9270E+05 | 4.8522E+05 |
| 1075 | C | 18 | 0.165 | 2.97 | 3.4649E+05 | 3.9139E+05 | 3.6894E+05 |
| 1200 | C | 18 | 0.104 | 1.872 | 5.8888E+05 | 5.3081E+05 | 5.5984E+05 |
| 1325 | C | 18 | 0.104 | 1.872 | 3.7520E+05 | 4.3213E+05 | 4.0366E+05 |
| 2520 | C | 18 | 0.131 | 2.358 | 3.6197E+05 | 3.7461E+05 | 3.6829E+05 |
| 0 | D | 3 | 0.04 | 0.12 | 2.5129E+04 | 4.0487E+04 | 3.2808E+04 |
| 790 | D | 16 | 0.059 | 0.944 | 2.5673E+05 | 2.9105E+05 | 2.7389E+05 |
| 970 | D | 10 | 0.227 | 2.27 | 4.4495E+05 | 4.5023E+05 | 4.4759E+05 |
| 1075 | D | 10 | 0.21 | 2.1 | 4.9924E+05 | 4.4823E+05 | 4.7373E+05 |
| 1200 | D | 10 | 0.127 | 1.27 | 4.8997E+05 | 4.1043E+05 | 4.5020E+05 |
| 1325 | D | 18 | 0.081 | 1.458 | 5.8311E+05 | 5.3521E+05 | 5.5916E+05 |
| 2520 | D | 18 | 0.084 | 1.512 | 5.8095E+05 | 5.8441E+05 | 5.8268E+05 |
| 0 | E | 3 | 0.047 | 0.141 | 3.4761E+04 | 1.4242E+04 | 2.4502E+04 |
| 790 | E | 8 | 0.147 | 1.176 | 2.7735E+05 | 2.2857E+05 | 2.5296E+05 |
| 970 | E | 10 | 0.247 | 2.47 | 4.6444E+05 | 4.5841E+05 | 4.6143E+05 |
| 1075 | E | 10 | 0.265 | 2.65 | 7.1468E+05 | 6.5304E+05 | 6.8386E+05 |
| 1200 | E | 10 | 0.154 | 1.54 | 6.7390E+05 | 7.6530E+05 | 7.1960E+05 |
| 1325 | E | 11 | 0.136 | 1.496 | 5.9944E+05 | 8.4989E+05 | 7.2467E+05 |
| 2520 | E | 10 | 0.182 | 1.82 | 6.8173E+05 | 7.2830E+05 | 7.0501E+05 |
| 0 | F | 3 | 0.041 | 0.123 | 1.5921E+04 | 3.9221E+04 | 2.7571E+04 |
| 790 | F | 8 | 0.121 | 0.968 | 3.4520E+05 | 2.8719E+05 | 3.1619E+05 |
| 970 | F | 10 | 0.242 | 2.42 | 4.6517E+05 | 4.0499E+05 | 4.3508E+05 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1075 | F | 10 | 0.198 | 1.98 | 4.9454E+05 | 4.2502E+05 | 4.5978E+05 |
| 1200 | F | 10 | 0.118 | 1.18 | 5.4818E+05 | 5.6125E+05 | 5.5472E+05 |
| 1325 | F | 10 | 0.115 | 1.15 | 4.4183E+05 | 4.9252E+05 | 4.6718E+05 |
| 2520 | F | 10 | 0.148 | 1.48 | 4.3443E+05 | 5.2238E+05 | 4.7841E+05 |

Table A3: Experiment 3 Raw Data

| Time | Flask | Dilution | Abs | Corrected Abs | Area 1 | Area 2 | Avg Area |
|---|---|---|---|---|---|---|---|
| 0 | A | 3 | 0.026 | 0.078 | 9.52412E+03 | 1.10109E+04 | 1.02675E+04 |
| 0 | B | 3 | 0.032 | 0.096 | 1.03272E+04 | 1.11613E+04 | 1.07443E+04 |
| 0 | C | 3 | 0.021 | 0.063 | 1.11138E+04 | 9.46819E+03 | 1.02910E+04 |
| 0 | D | 3 | 0.018 | 0.054 | 1.45232E+04 | 1.07742E+04 | 1.26487E+04 |
| 0 | E | 3 | 0.022 | 0.066 | 1.22140E+04 | 9.38738E+03 | 1.08007E+04 |
| 0 | F | 3 | 0.019 | 0.057 | 1.03722E+04 | 1.24427E+04 | 1.14075E+04 |
| 130 | A | 3 | 0.029 | 0.087 | | | |
| 130 | B | 3 | 0.035 | 0.105 | | | |
| 130 | C | 3 | 0.031 | 0.093 | | | |
| 130 | D | 3 | 0.02 | 0.06 | | | |
| 130 | E | 3 | 0.023 | 0.069 | | | |
| 130 | F | 3 | 0.022 | 0.066 | | | |
| 250 | A | 3 | 0.058 | 0.174 | 1.78128E+04 | 1.07643E+04 | 1.42886E+04 |
| 250 | B | 3 | 0.067 | 0.201 | 1.29752E+04 | 1.32443E+04 | 1.31098E+04 |
| 250 | C | 3 | 0.063 | 0.189 | 9.22814E+03 | 8.74381E+03 | 8.98598E+03 |
| 250 | D | 3 | 0.033 | 0.099 | 9.93513E+03 | 1.21125E+04 | 1.10238E+04 |
| 250 | E | 3 | 0.035 | 0.105 | 1.18795E+04 | 6.49746E+03 | 9.18848E+03 |
| 250 | F | 3 | 0.035 | 0.105 | 6.61523E+03 | 1.05336E+04 | 8.57442E+03 |
| 385 | A | 3 | 0.17 | 0.51 | 1.63152E+04 | 1.37649E+04 | 1.50401E+04 |
| 385 | B | 3 | 0.178 | 0.534 | 1.49557E+04 | 2.50697E+04 | 2.00127E+04 |
| 385 | C | 3 | 0.177 | 0.531 | 2.75014E+04 | 2.67064E+04 | 2.71039E+04 |
| 385 | D | 3 | 0.057 | 0.171 | 3.21404E+04 | 1.77974E+04 | 2.49689E+04 |
| 385 | E | 3 | 0.06 | 0.18 | 1.67839E+04 | 1.78590E+04 | 1.73215E+04 |
| 385 | F | 3 | 0.066 | 0.198 | 1.97082E+04 | 2.66662E+04 | 2.31872E+04 |
| 500 | A | 6 | 0.207 | 1.242 | 9.63544E+03 | 1.78099E+04 | 1.37227E+04 |
| 500 | B | 6 | 0.206 | 1.236 | 2.29226E+04 | 1.73316E+04 | 2.01271E+04 |
| 500 | C | 6 | 0.206 | 1.236 | 9.28056E+03 | 2.26545E+04 | 1.59675E+04 |
| 500 | D | 3 | 0.086 | 0.258 | 1.93416E+04 | 2.97073E+04 | 2.45245E+04 |
| 500 | E | 3 | 0.085 | 0.255 | 1.35420E+04 | 2.46699E+04 | 1.91060E+04 |
| 500 | F | 3 | 0.087 | 0.261 | 2.01210E+04 | 6.29347E+03 | 1.32072E+04 |
| 620 | A | 10 | 0.226 | 2.26 | 2.70344E+04 | 8.61377E+03 | 1.78241E+04 |
| 620 | B | 10 | 0.194 | 1.94 | 0.00000E+00 | 3.53408E+04 | 1.76704E+04 |
| 620 | C | 10 | 0.173 | 1.73 | 3.13643E+04 | 2.48246E+04 | 2.80945E+04 |
| 620 | D | 3 | 0.115 | 0.345 | 1.13838E+04 | 7.10440E+03 | 9.24410E+03 |
| 620 | E | 3 | 0.105 | 0.315 | 1.11881E+04 | 1.76565E+04 | 1.44223E+04 |
| 620 | F | 3 | 0.113 | 0.339 | 1.29814E+04 | 3.81548E+04 | 2.55681E+04 |
| 1400 | A | 14 | 0.216 | 3.024 | 1.65422E+04 | 2.23620E+04 | 1.94521E+04 |
| 1400 | B | 14 | 0.194 | 2.716 | 2.57875E+04 | 6.59937E+03 | 1.61934E+04 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1400 | C | 14 | 0.195 | 2.73 | 1.88716E+04 | 2.17482E+04 | 2.03099E+04 |
| 1400 | D | 6 | 0.261 | 1.566 | 0.00000E+00 | 8.69823E+03 | 4.34912E+03 |
| 1400 | E | 6 | 0.192 | 1.152 | 1.96573E+04 | 2.17074E+04 | 2.06824E+04 |
| 1400 | F | 6 | 0.135 | 0.81 | 3.76641E+04 | 2.53214E+04 | 3.14928E+04 |
| 1675 | A | 14 | 0.24 | 3.36 | 2.90957E+04 | 2.77373E+04 | 2.84165E+04 |
| 1675 | B | 14 | 0.207 | 2.898 | 2.86723E+04 | 1.25055E+04 | 2.05889E+04 |
| 1675 | C | 14 | 0.211 | 2.954 | 1.93731E+04 | 2.17316E+04 | 2.05524E+04 |
| 1675 | D | 8 | 0.234 | 1.872 | 1.04733E+04 | 1.92078E+04 | 1.48406E+04 |
| 1675 | E | 8 | 0.157 | 1.256 | 1.98411E+04 | 1.61110E+04 | 1.79761E+04 |
| 1675 | F | 8 | 0.119 | 0.952 | 2.39397E+04 | 2.74432E+04 | 2.56915E+04 |

Table A4: Experiment 4 Raw Data

| Time | Flask | Dilution | Abs | Corrected Abs | Area 1 | Area 2 | Avg Area |
|---|---|---|---|---|---|---|---|
| 0 | A | 4 | 0.037 | 0.148 | 2.27552E+04 | 2.52468E+04 | 2.40010E+04 |
| 0 | B | 4 | 0.038 | 0.152 | 2.34963E+04 | 1.73782E+04 | 2.04373E+04 |
| 0 | C | 4 | 0.036 | 0.144 | 2.64155E+04 | 3.42807E+04 | 3.03481E+04 |
| 0 | D | 4 | 0.030 | 0.12 | 1.37378E+04 | 2.53479E+04 | 1.95429E+04 |
| 0 | E | 4 | 0.025 | 0.1 | 1.72987E+04 | 4.46414E+04 | 3.09701E+04 |
| 0 | F | 4 | 0.023 | 0.092 | 3.67985E+04 | 3.13896E+04 | 3.40941E+04 |
| 135 | A | 4 | 0.039 | 0.156 | 3.00791E+04 | 4.33652E+04 | 3.67222E+04 |
| 135 | B | 4 | 0.044 | 0.176 | 4.63234E+04 | 3.50169E+04 | 4.06702E+04 |
| 135 | C | 4 | 0.045 | 0.18 | 5.91663E+04 | 6.71292E+04 | 6.31478E+04 |
| 135 | D | 4 | 0.036 | 0.144 | 3.26454E+04 | 1.47182E+04 | 2.36818E+04 |
| 135 | E | 4 | 0.033 | 0.132 | 2.42199E+04 | 2.79093E+04 | 2.60646E+04 |
| 135 | F | 4 | 0.033 | 0.132 | 2.52584E+04 | 1.97976E+04 | 2.25280E+04 |
| 225 | A | 4 | 0.034 | 0.136 | 3.01786E+04 | 3.35689E+04 | 3.18738E+04 |
| 225 | B | 4 | 0.047 | 0.188 | 3.21309E+04 | 4.27197E+04 | 3.74253E+04 |
| 225 | C | 4 | 0.043 | 0.172 | 5.42012E+04 | 4.93622E+04 | 5.17817E+04 |
| 225 | D | 4 | 0.020 | 0.08 | 1.69458E+04 | 1.94781E+04 | 1.82120E+04 |
| 225 | E | 4 | 0.022 | 0.088 | 3.06118E+04 | 3.12453E+04 | 3.09286E+04 |
| 225 | F | 4 | 0.020 | 0.08 | 2.67421E+04 | 4.22461E+04 | 3.44941E+04 |
| 360 | A | 4 | 0.051 | 0.204 | 2.20667E+04 | 2.34617E+04 | 2.27642E+04 |
| 360 | B | 4 | 0.100 | 0.4 | 7.64535E+04 | 8.20171E+04 | 7.92353E+04 |
| 360 | C | 4 | 0.113 | 0.452 | 1.12506E+05 | 1.02341E+05 | 1.07424E+05 |
| 360 | D | 4 | 0.024 | 0.096 | 3.11680E+04 | 4.15264E+04 | 3.63472E+04 |
| 360 | E | 4 | 0.028 | 0.112 | 4.62284E+04 | 3.58087E+04 | 4.10186E+04 |
| 360 | F | 4 | 0.030 | 0.12 | 4.95208E+04 | 5.14017E+04 | 5.04613E+04 |
| 480 | A | 4 | 0.073 | 0.292 | 2.30097E+04 | 1.24721E+04 | 1.77409E+04 |
| 480 | B | 8 | 0.101 | 0.808 | 8.29353E+04 | 6.91218E+04 | 7.60286E+04 |
| 480 | C | 4 | 0.230 | 0.92 | 9.98401E+04 | 8.37599E+04 | 9.18000E+04 |
| 480 | D | 4 | 0.033 | 0.132 | 3.61997E+04 | 3.26995E+04 | 3.44496E+04 |
| 480 | E | 4 | 0.067 | 0.268 | 6.85592E+04 | 5.18496E+04 | 6.02044E+04 |
| 480 | F | 4 | 0.070 | 0.28 | 6.63198E+04 | 9.31909E+04 | 7.97554E+04 |
| 665 | A | 4 | 0.120 | 0.48 | 1.51330E+04 | 2.81833E+04 | 2.16582E+04 |
| 665 | B | 4 | 0.209 | 0.836 | 7.10874E+04 | 5.94965E+04 | 6.52920E+04 |
| 665 | C | 4 | 0.238 | 0.952 | 1.39508E+05 | 1.30522E+05 | 1.35015E+05 |
| 665 | D | 4 | 0.020 | 0.08 | 2.45214E+04 | 3.43466E+04 | 2.94340E+04 |
| 665 | E | 4 | 0.081 | 0.324 | 8.23113E+04 | 9.82056E+04 | 9.02585E+04 |
| 665 | F | 4 | 0.119 | 0.476 | 1.54499E+05 | 1.61480E+05 | 1.57990E+05 |
| 1200 | A | 4 | 0.217 | 0.868 | 1.52274E+04 | 6.34819E+03 | 1.07878E+04 |
| 1200 | B | 8 | 0.255 | 2.04 | | | |
| 1200 | C | 8 | 0.256 | 2.048 | 8.07992E+04 | 6.68932E+04 | 7.38462E+04 |
| 1200 | D | 4 | 0.025 | 0.1 | 2.62145E+04 | 2.61004E+04 | 2.61575E+04 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1200 | E | 4 | 0.086 | 0.344 | 6.57520E+04 | 7.92627E+04 | 7.25074E+04 |
| 1200 | F | 4 | 0.133 | 0.532 | 1.33340E+05 | 1.38150E+05 | 1.35745E+05 |

# Appendix B: Rate Determination Figures

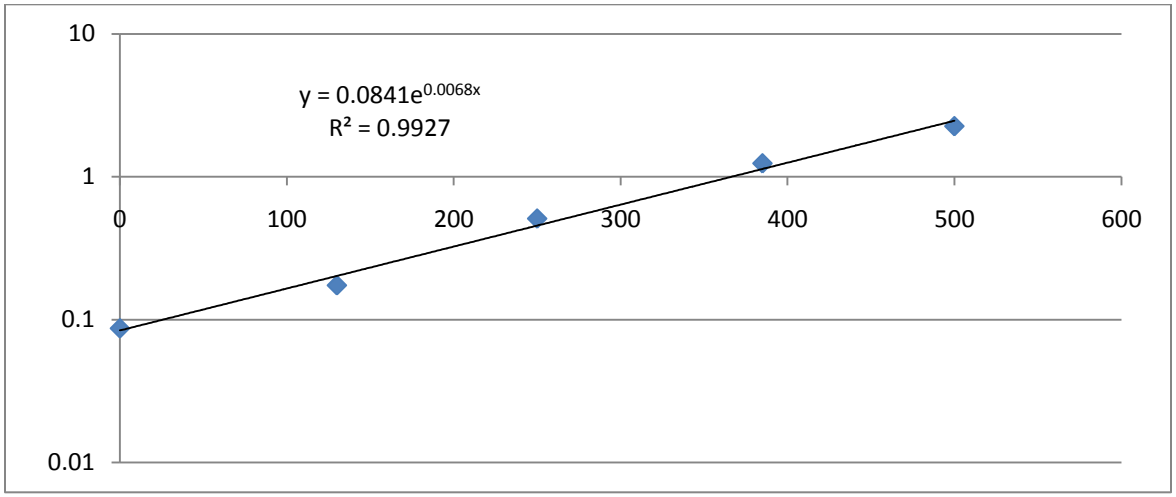


Figure B1: Reaction rate for Wild Yeast with all Glucose

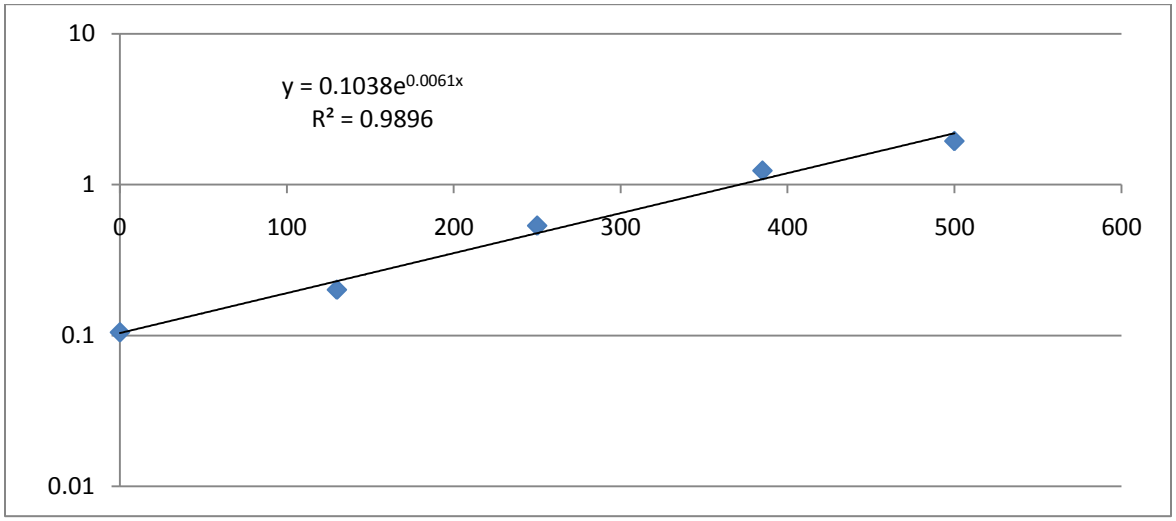


Figure B2: Reaction rate for Wild Yeast with 6g/L Glucose and 4g/L Xylose

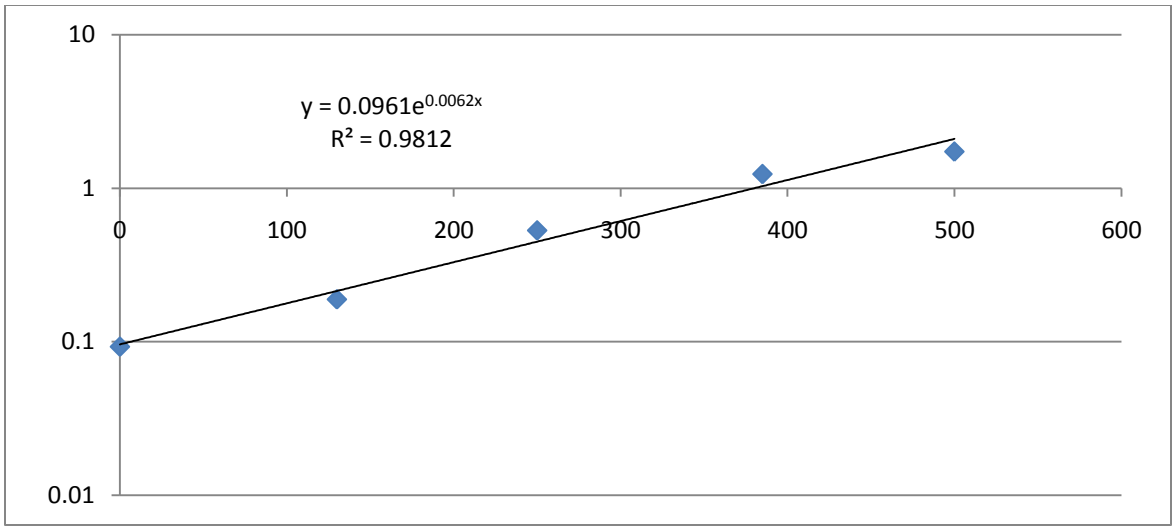


Figure B3: Reaction rate for Wild Yeast with 4g/L Glucose and 6g/L Xylose

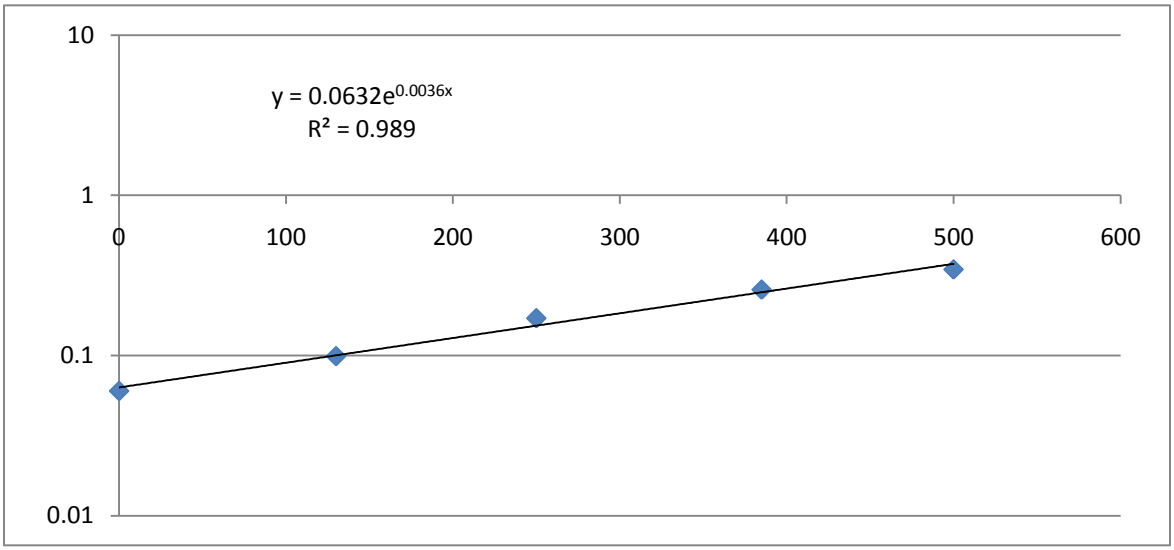


Figure B4: Reaction rate for Modified Yeast with all Glucose

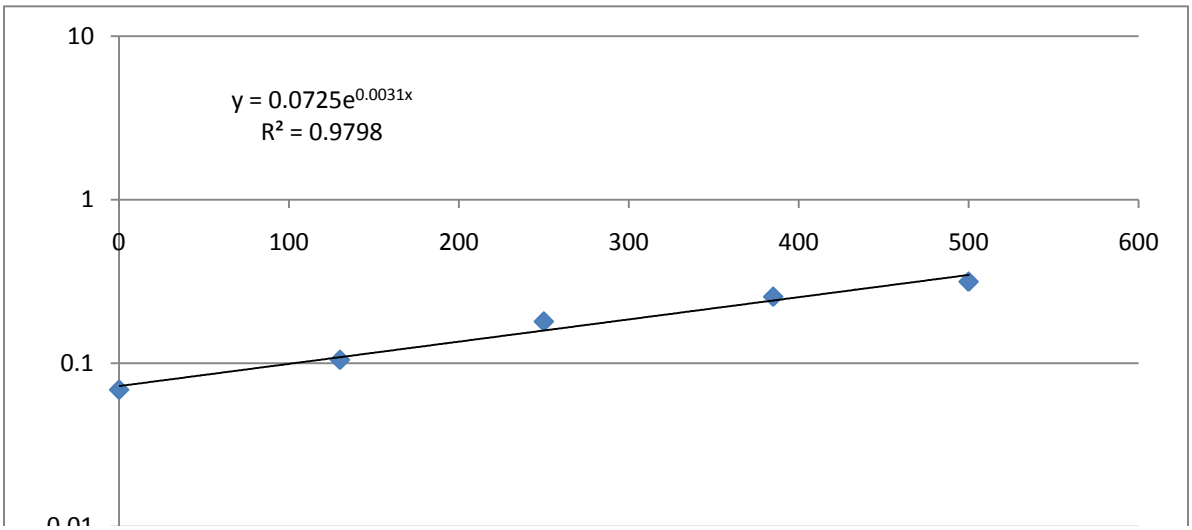


Figure B5: Reaction rate for Modified Yeast with 6g/L Glucose and 4g/L Xylose

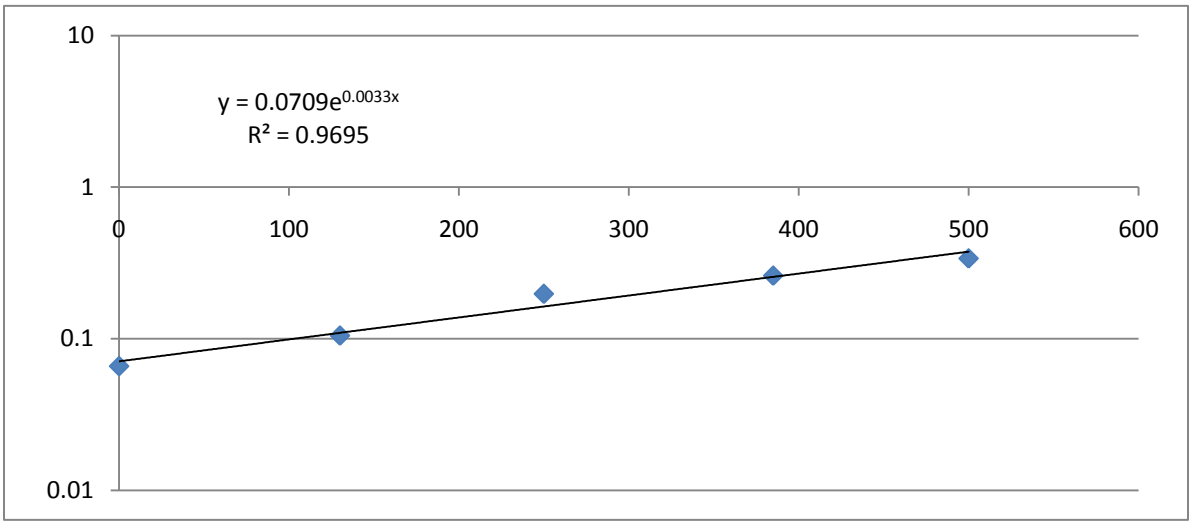


Figure B6: Reaction rate for Modified Yeast with 4g/L Glucose and 6g/L Xylose