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Determining the Effect of Chemical and Physical Pretreatments on the Yield and Energy Output of Cellulosic Ethanol from *Panicum Virgatum*

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Summary

The world needs an energy source that can replace nonrenewable fossil fuels, which release greenhouse gases into the atmosphere. We explored the production of ethanol from switchgrass, a North American grass that is suitable for biofuel production because it contains large amounts of cellulose, a chemical energy that can be used as fuel. Our experiments evaluated whether key factors would increase the efficiency of ethanol production from switchgrass—in other words, which factors produced the highest percentage of ethanol in the final step of distillation. There are four steps to producing ethanol: pretreatment, enzymatic hydrolysis, fermentation, and distillation. We tested two variables: the concentration of potassium hydroxide (KOH) added to the switchgrass during pretreatment and using ground versus unground grass. After adding the KOH to the grass and letting it sit for a week, we balanced the pH and added 5 grams of cellulase to each sample to convert the cellulose to glucose. After 48 hours, we added 10 grams of yeast to turn the glucose into ethanol. In the final step, we distilled all of the samples and measured the percentage of alcohol. Most large-scale production companies use a 30% KOH concentration during their pretreatment procedure, leading us to speculate that a 30% KOH concentration would yield the greatest amount of ethanol. Contrary to this expectation, we found that the sample with 15% KOH content produced the greatest ethanol yield.

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Introduction

As fossil fuels like oil, coal, and natural gas are finite, there is a danger of them running out. How quickly they will run out depends on how much of these resources are left and how quickly they are being used. Consequently, a new source of energy must emerge in order to secure the future of energy. Biomass energy is an important source of clean, renewable energy. The production of biofuels requires biological sources, such as trees and plants, that can be harvested for their energy. The carbon released by biofuel when burned is similar to the amount of carbon taken in by the plant during photosynthesis. As a result, there is a reduced effect on atmospheric carbon dioxide levels relative to burning fossil fuels. Fossil fuels contribute to the release of heat-trapping gases, like carbon dioxide, which absorb heat and keep it trapped on the Earth's surface (1). The resulting "greenhouse effect" increases temperature, melts ice caps and glaciers, and causes rapid climate change.

Fortunately, there are a vast number of biomass sources, like plants, that can sustainably produce ethanol. During the process of photosynthesis, the chlorophyll in plants captures the sun's energy and converts it into cellulose, the most abundant organic polymer on Earth. Cellulose is a polysaccharide containing linked glucose units (2). Producing ethanol from cellulose requires breaking down the cellulose into glucose in a process known as hydrolysis. In fermentation, sugars are converted into cellular energy and ethanol is produced as a waste product.

Beneficial biomasses should be renewable and provide sources of low-carbon energy. These biomasses include energy crops that do not compete with food crops – in this case, switchgrass. *Panicum virgatum*, or switchgrass, grows all over the United States: in the Great Plains, the Midwest, and the South. Using switchgrass as an energy source therefore helps reduce dependency on foreign nations for fuel. Switchgrass is extremely hardy; it is resistant to floods, droughts, nutrient-poor soils, and pests (3). Unlike other plants, switchgrass does not require fertilizer for maximum growth. It is large and tough, and grows up to 10 feet tall while using water efficiently. In addition, switchgrass ethanol delivers 540% of the energy used to produce it, compared with just roughly 25% more energy returned by corn-based ethanol, according to the most optimistic studies (3).

Lignocellulosic biomass contains polymers of cellulose, hemicellulose, and lignin bound together in a complex structure (4). Before ethanol can be produced from the cellulose, switchgrass must be pretreated to set the cellulose free. Without pretreatment, the tightly bound structure can hinder the hydrolysis of cellulose. There are several different ways to pretreat switchgrass: alkali pretreatment, ammonia pretreatment, acid pretreatment, etc. However, from previous research, we found that using potassium hydroxide to pretreat grass produced the greatest amount of ethanol. Potassium hydroxide (KOH) was chosen due to its lower cost in comparison to similar chemicals. Furthermore, it creates a greater overall ethanol yield in comparison to acid pretreatments. Therefore, we used only one chemical but varied the concentrations of the KOH: 0% (the control), 5%, 10%, 15%, 20%, and 30%. Modern large-scale production companies typically use a 30% concentration of potassium hydroxide (or other chemicals) in order to pretreat biomass. Our objective was to determine if the same industrial ethanol yield and level of efficiency could be reached with a lower KOH concentration, which would result in the use of fewer chemicals. As a result, there would be less of a detrimental environmental impact and a lower cost for chemicals.

Another variable was also physically grinding the grass to break it down into smaller particles for one set, while leaving the other set physically untreated. In total, we had 12 samples, each either unground or ground with a variable KOH concentration.

The switchgrass we used underwent the four necessary steps of ethanol production: pretreatment, enzymatic hydrolysis, fermentation, and distillation. The variables icluded the pretreatment of the individual samples: the concentration of potassium hydroxide added (0%, 5%, 10%, 15%, 20%, 30%) and ground versus unground grass. We predicted that the highest concentration of KOH at 30% and adding physical pretreatment by grinding the switchgrass into finer particles would yield the highest percentage of ethanol due to greater exposure of alkali salts to the lignin in order to degrade its structure.

During the four processes, we attempted to increase the overall energy output by decreasing the amount of energy put into ethanol generation. Common pretreatment methods require autoclaving samples and employing an incubator. We eliminated that energy by only utilizing the distillation apparatus to purify our samples. Therefore, we determined whether the energy we produced was enough to create a positive energy output, including the subtraction of energy for distilling. First, we used the dry, leftover solid residue to determine its potential energy. In large-scale production, the solid byproduct would be re-used for more ethanol, not disposed of. To determine the potential energy, we made a calorimeter and determined the kJ of energy that would be produced if it were re-used. In addition, ethanol produces 21.2 kJ/mL. Using this figure, we were able to determine the kJ of energy produced if the ethanol were used. By combining the potential energy of the residue and the energy from the ethanol, we were able to determine the total energy output.

Results

During our experiment, we conducted two different tests with various conditions. We conducted two trials for each variable. **Figures 1 and 2** show the averages of both trials. When conducting statistical analysis, we used a T-test for two different groups measured twice on one variable. We compared the glucose content and ethanol percentages in the presence or absence of added sugar for all samples. We calculated a p-value of 0.02932 for the glucose samples and a p-value of 0.002264 for the ethanol percentages. Since the p-value was significantly less than 0.05 for both, we reject the null hypothesis, indicating a statistically reliable mean difference between the results of both tests in glucose content and percentage of ethanol.

Table 1 shows the average of the trials for the two different tests when we determined the total energy produced in kJ. We used an unpaired T-test for two different groups measured twice on one variable. We compared the energy values in the absence or presence of added sugar. We calculated a p-value of 0.000312 indicating a statistically reliable mean difference between the results of both tests in energy output.

Discussion

In the first phase of the project, the addition of sugar during the process of fermentation drastically increased the concentration of ethanol. The highest ethanol concentration, 20.99%, was yielded by a combination of 15% chemical pretreatment and the addition of physical pretreatment (grinding of the grass) (**Figure 1**). The highest percent yield was 7.79% without added sugar, and was produced by a 15% KOH concentration (**Figure 2**). However, the grinding of the grass did not make a significant difference in the production of ethanol. In fact, unground grass performed better than the ground grass in the cases of 5%, 20%, and 30%. It may take further investigation, but it seems as though physical pretreatment contributes little. A potential cause of lower ethanol yields in, for example, the “Unground 15%” sample (**Figure 1**), could have been an issue with the yeast. Some of the lower values of ethanol suggest that the yeast could have died before reaching a higher concentration. In addition, the presence of alkali salts and sulfuric acid could have hindered the growth of the cellulase and yeast. Besides this source of error, another possible explanation is that the glucose contents were lower in the samples with lower ethanol yields, causing a smaller amount of sugar to be fermented into ethanol.

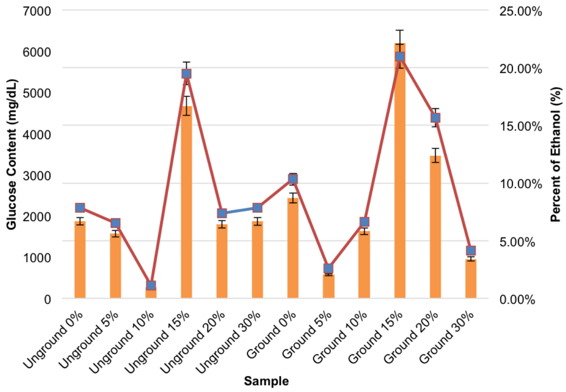

Figure 1: Comparison of glucose and ethanol content with added sugar. This trial included the addition of sugar in order to activate the yeast in the fermentation stage. After the hydrolysis of the samples occurred, a blood glucose meter was used to determine the level of glucose in each sample in mg/dL. These values are represented by the green bars. Once the glucose in the samples was fermented using distillers' yeast, the ethanol concentrations were determined using specific gravity calculations. The ethanol percentages are represented by the blue background of the graph. Error bars indicate standard error of the mean.

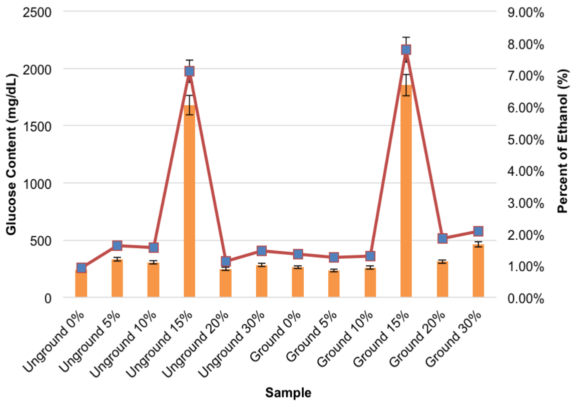

Figure 2: Comparison of glucose and ethanol content without added sugar. This trial excluded the addition of sugar in order to activate the yeast in the fermentation stage. After the hydrolysis of the samples occurred, a blood glucose meter was used to determine the level of glucose in each sample in mg/dL. The values are represented by the green bars. Once the glucose in the samples was fermented using distillers' yeast, the ethanol concentrations were determined using specific gravity calculations. The ethanol percentages are represented by the blue background of the graph. Error bars indicate the standard deviation of each of the samples.

"Ground 15%" produced the highest glucose content in the hydrolysis step at 1854 mg/dL (**Figure 2**), showing that glucose content and ethanol yield have a positive correlation, even with the exclusion of added sugar in Test 2. Ground 15% produced the highest total energy of 16,982 kJ (**Table 1**).

Additionally, the addition of sugar correlated with the higher ethanol percentages seen in Test 1. It is possible to produce a 7% ethanol concentration without any sugar to activate the yeast. We conclude that a 15% KOH concentration for pretreatment will yield the highest ethanol yield and after further research, should be looked into as a possibility for large-scale production instead of using other highly concentrated chemicals. Without any added sugar, a 15% KOH concentration can produce 16,983 kJ of energy, a positive energy output. A 15% KOH concentration consistently performed better, likely due to the fact that it contained enough alkali salts to break down the lignin but not so much as to hinder the growth of the microorganisms like the cellulase and yeast. When the sulfuric acid was added to bring the pH to approximately 6.75, the samples containing a higher KOH concentration – like 20% and 30% – required more acid to neutralize the pH. As a result, the presence of a large amount of acid and alkali salts likely hindered the growth of cellulose and yeast necessary to hydrolyze and ferment the cellulose into ethanol. For the control, 5%, and 10% conditions, there were likely not enough KOH salts to initially degrade the lignin during pretreatment. The middle concentration of 15% likely had just enough salts to target the lignin but not enough acid and salts to act as a barrier for the growth of the cellulase and yeast. As a result, these conditions aided the cellulase in hydrolysis and the yeast in fermentation of more glucose molecules to create more ethanol.

For future studies, determining the exact kilojoules of energy required for distillation and subtracting that value from the energy output is a potential next step. By doing so, it would be possible to determine if ethanol production would have a positive net energy output, strongly suggesting energy efficiency.

Methods

Pretreatment

One hundred grams of switchgrass (dried and cut into 2-inch long pieces) and 5 g of potassium hydroxide pellets (reagent grade) were measured out. Depending on the concentration of KOH desired, a certain amount of pellets was used. For instance, 5 g was used for a 5% concentration of KOH and 10 g was used for a 10% KOH concentration. The pellets were dissolved into 1 L of water. Half of the samples requiring physical pretreatment were placed into an electric food processor and broken down into smaller particles. The KOH solution and grass were combined and allowed to sit for one week at room temperature.

Hydrolysis

Hydrolyzing the cellulose involves breaking down

Sample	Energy of Ethanol (kJ/kg)		Energy of Residue (kJ)		Total Energy (kJ)	
	with sugar	without sugar	with sugar	without sugar	with sugar	without sugar
Unground 0%	33,326	1,950	845	845	34,171	2,795
Unground 5%	27,645	3,434	688	688	28,333	4,122
Unground 10%	4,579	3,350	535	535	5,114	3,885
Unground 15%	82,680	15,094	483	483	83,163	15,577
Unground 20%	31,291	2,417	461	461	31,752	2,878
Unground 30%	33,326	3,116	523	523	33,849	3,639
Ground 0%	43,757	2,883	832	832	44,589	3,715
Ground 5%	10,939	2,671	866	866	11,026	3,537
Ground 10%	28,238	2,756	453	453	28,691	3,209
Ground 15%	89,040	16,494	489	489	89,529	16,983
Ground 20%	66,398	3,943	271	271	66,669	4,214
Ground 30%	17,554	4,410	234	234	17,788	4,644

Table 1: Energy output with and without added sugar. The energy of ethanol was determined in kJ by multiplying the energy in 1 mL of ethanol by the total volume of ethanol found in each of the samples. The energy of the residue was found by using a calorimeter to determine the potential energy of the leftover residual grass. The total energy was found by combining the energy of the ethanol and the potential energy of the residue. Overall, the energy was measured in kilojoules. The table shows the average of the energy values from the two trials performed with sugar and without added sugar.

the polysaccharide cellulose into monosaccharide units. The reaction is catalyzed by cellulase. Produced chiefly by fungi, bacteria, and protozoans that engage in cellulolysis, the powder form we used contained 75,000 cellulase units per gram, according to Carolina Biological Supply (5). The optimum pH for cellulase is 6.5 to 7.0, so we added sulfuric acid to the samples to bring them down to a pH of 6.75 ± 0.02. We determined how much acid to add using the function: $pH = -\log [H+]$ and the equation $M1V1 + M2V2 = M3(V1 + V2)$. Using a pH meter, sulfuric acid (18 M or 95–98% v/v) was added in 1-mL increments until the pH reached 6.75 ± 0.02, the optimum pH level for cellulase. Afterwards, we added 5 g of powdered cellulase to each sample and stored them at room temperature for a week. Afterwards, a blood glucose meter was used to determine the presence of glucose and measure the glucose content in mg/dL.

Fermentation

Once the cellulase has converted the cellulose into glucose molecules, the glucose units should undergo alcoholic fermentation, the process of converting sugar into ethanol. We added distillers' yeast, which has the highest alcohol tolerance at 21%, according to Midwest Supplies (6). 240 g of yeast was added to 4.8 kg of sugar and 24 L of warm water to activate the yeast. Two liters of the resulting solution was added to each of the samples, so each received 20 g of yeast and 400 g of sugar to activate the yeast. We kept the samples at 21°C, or room temperature for a week; aerobic fermentation takes 24-48 hours. The exclusion of an incubator for temperature control also reduced the amount of energy input, which by definition, increased efficiency.

Distillation

Distillation is an act of purification; although it may not be part of the chemical process of ethanol production, when ethanol is produced for commercial use, it is necessary. If the solution produced were to be put in a vehicle without distillation, the impurities and chemicals from the previous steps would be detrimental. We added 500 mL from each sample to a distillation flask and attached the flask to a distillation apparatus. The solutions were boiled in a distillation apparatus so that their vapor would rise, hit cold water in a cylinder, and condense. Each sample was distilled until either 400 mL of distillate was collected or the temperature of the sample surpassed 78.4°C, the boiling point of ethanol. Once the temperature passed this point, other substances besides ethanol – for instance, extra water – were bound to collect with the distillate.

Determining Ethanol Yield

In order to determine the overall ethanol yield, we employed calculations involving specific gravity, the ratio of density of a material to the density of water at a given temperature, where density is defined as the material's mass per unit volume and is measured in kg/m^3. The equation $SG = p/pW$ where SG = specific gravity, p = density of the material (kg/m^3), and pW = density of water (kg/m^3) was used to determine ethanol percentages. Exactly 100 mL of the distillate was carefully poured into a volumetric flask, and the total weight was recorded. The weight of the flask was subtracted from the total weight. The resulting g/100 mL density measurement was converted into kg/m^3. ($1 kg/m^3 = 1 g/L$) and divided by the density of water, 999.97 kg/m^3. The resulting number was used to calculate ethanol concentration. The following variables were used: x = unknown volume of water and (1-x) = unknown volume of alcohol. Then x + (1-x) = 1 Liter. The specific gravity of water is 1.0, and the specific gravity of ethanol is 0.785. The equation (x)(1.0) + (1-x)(0.785) = Sp. G of solution was used. By solving for x, we used the following to determine ethanol content: (x)(100%) = the concentration of water, and (1-x)(100%) = the concentration of alcohol.

Net Energy Output

A small can was filled with 100 grams of water. Five grams of the dry, solid residue was measured and put in a crucible. Two large holes were cut into the side of a large can and placed on top of the crucible. The small can was suspended on top of the larger can. The initial temperature of the water was measured with a thermometer and recorded. Using a Bunsen burner, the grass was completely burned, and the maximum temperature that the water reached was recorded. After waiting for the remaining grass to cool to room temperature, the grass was weighed. Changes in mass of the grass, temperature, and the mass of water were determined before and after burning the grass. The equation $q = mC\Delta T$ (with q as the energy in joules, m as the mass of water, C as the specific heat of water (4.186 joule/gram °C), and ΔT as the change in temperature) was used. The volume of ethanol the distillate contained was determined by multiplying the concentration of ethanol (found in the previous section) by the milliliters of total distillate. The total volume of ethanol in mL per 100 g (original sample) was then converted to mL/kg. Using the value 21.2 kJ/mL, the kJ of energy in the ethanol was determined by multiplying the value by the milliliters of ethanol.

Removing Sugar in Fermentation (Test 2)

During our first phase, we added sugar to activate the yeast. However, we suspected that the sugar was creating the high percentages of ethanol instead of the cellulose. Therefore, in the second phase, we used the glucometer to measure the glucose content. Since it showed the presence of sugar, we did not add any additional sugar to see if the yeast would be able to produce ethanol by only using the sugar from the cellulose. To conduct Test 2, all the previous procedures were followed. Instead of adding sugar in the fermentation stage, 20 g of the distiller's yeast was directly added to each sample and stirred until dissolved.

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