

Enhancing Ethanol Production from Biomass through Organic Acid Pretreatment: A Sustainable Alternative to Fossil Fuels

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Abstract

Usage of fossil fuels has been a vital energy source powering everyday life. Excessive combustion of fossil fuels has led to an increase in greenhouse gas emissions subsequently increasing global temperatures. The purpose of this experiment was to test different pretreatment methods (organic acids, alkali, and heat) and compare their effectiveness during the fermentation process as a substitute for fossil fuels. The experiment was conducted using 2 grams of ground wood chips, grass and 8 grams of grounded corn stover. Then, mixtures of each level of the independent variable were made by adding each biomass sample with acid- water, alkali-water, and boiled water solutions. Each solution was tested for glucose levels and subsequently yeast fermentation was carried out in control group and test group when ethanol levels were measured. Based upon the collected data of the glucose and ethanol measurements for each solution during every step in the process, the main finding of the research was that organic acids, namely oxalic acid, was the most effective in deriving the most plant sugars needed for yeast fermentation of the biomass process. Organic acid pretreatment proved to be more viable compared to just heat or alkali pretreatment in the biomass processing. The hypothesis was supported by this experiment as corn husk treated with oxalic acid had produced the highest ethanol yield. The data collected shows corn husk control produced 2.450% ethanol yields on average and oxalic acid treated corn husk produced 2.460% ethanol on average (the units for the percentages were in percent of solution). The implications of this research prove that organic acid treated biomass produces significant amounts of ethanol during the fermentation process. In addition to being safer to handle, organic acids can improve the cost/benefit analysis of the fermentation process through reducing the overall inhibitor production leading to a higher overall yield for a bargained price.

Introduction

Usage of fossil fuels has been a vital energy source powering everyday life. Excessive combustion of fossil fuels has led to an increase in greenhouse gas emissions subsequently increasing global temperatures. Global Warming itself has many negative outcomes for the environment such as increasing water levels and more frequent and severe weather patterns. With arising concerns, alternative fuel sources are actively being researched to find a substitute for the ever-depleting fossil fuels. Biomass refers to any living or once living organic material containing sugars that can be harvested as an energy source. Therefore, exploration of lignocellulosic raw material for biofuel production is also considered to be a significant issue. Experimenting on a biomass sample that is rich in cellulosic (plant sugar) content such as corn, produces the most fuel. A crucial step in the biomass process which can vary in effectiveness is its pretreatment step.

In the pretreatment process, the biomass sample is subjected to various treatment methods such as application of acids, alkalis, or high heat to make the sugars in the plant's cell wall more accessible. Pretreatment is an important step in the processing therefore, researchers are currently looking into a way to most effectively derive the sugar without harming any other essential plant nutrients. In this experiment, organic acids (Citric and Oxalic) were tested in comparison to commercially used, concentrated sulfuric acid. Although concentrated sulfuric acid has been used by many federal biomass organizations, there is evidence that dilute and organic acids may have potential in the industry. For one, usage of dilute or organic acids is more cost-effective. Next, it is not as

corrosive as concentrated acids which require expensive corrosive resistant reactors. Lastly, the organic acids produce less inhibitory byproducts. If the type of biomass sample and pretreatment method is related to the ethanol yields when fermented and can be tested by using various biomass samples (corn husk, grass, and woodchips) and subjecting them to different pretreatment types (acid, alkali, and heat) and finally, comparing their ethanol yields, then corn husk with oxalic acid pretreatment would produce the highest yields. This will happen because corn husk's high lignocellulosic content paired with oxalic acid's ability to effectively free up cellulose would allow the most glucose to be converted from cellulose and eventually into ethanol during yeast fermentation.

Methods

Materials

For this experiment, the three different biomass samples tested were corn stover, grass, and woodchips. The three levels of the independent variable were Citric Acid, Oxalic Acid, and Calcium Hydroxide. The control for this experiment was corn stover without any chemical pretreatment method. The materials used in conducting this experiment were: 8 grams of ground corn stover, 2 grams of ground woodchips, and 2 grams of ground grass for each trial. In addition to the biomass sample, 2 grams of each of the levels of the independent variable were needed along with 750mL of distilled water. Specific measurement and procedural tools that were used in this experiment included a standard blood glucometer, Vernier Ethanol sensor with a probe, a common water bath, a test tube rack, the cellulase enzyme, and the standard baker's yeast.

The Effect of Different Biomass Samples on Glucose/Ethanol Yields When Fermented																				
Biomass Sample	Glucose/Ethanol Yields (mg/dL and atmospheric %)																			
	Pre-Heat Treatment Glucose Levels (mg/dL)					Post-Heat Treatment Glucose Levels (mg/dL)					Post-Enzymatic Hydrolysis Glucose Levels (mg/dL)					Ethanol Yields (%)				
	Trial 1	Trial 2	Trial 3	Average	Standard Deviation	Trial 1	Trial 2	Trial 3	Average	Standard Deviation	Trial 1	Trial 2	Trial 3	Average	Standard Deviation	Trial 1	Trial 2	Trial 3	Average	Standard Deviation
Grass	46	38	46	43	4.62	88	85	182	118	55.16	401	482	447	443	40.62	1.567%	2.169%	1.654%	1.797%	0.33%
Woodchips	error 6	error 6	error 6	error 6	error 6	<40	52	<40	44	6.93	282	494	382	386	106.06	2.429%	1.390%	2.438%	2.086%	0.60%
Corn Husk	287	192	219	233	48.95	371	398	240	336	84.51	514	485	325	441	101.79	2.370%	2.008%	2.973%	2.450%	0.49%

Fig. 1. This is the descriptive data table for glucose and ethanol levels of each of the different biomass samples, corn husk, woodchips, and grass. The averages are displayed as averages in bold. As displayed by the data, Corn husk had by far the most ethanol and was a close second to the glucose produced by grass. The ethanol levels go as follows, corn husk with 2.45%, woodchips with 2.086%, and grass with 1.797%.

The Effect of Different Pretreatment Methods on Glucose/Ethanol Yields When Fermented																		
Acid/Alkali Type	Glucose/Ethanol Yields (mg/dL and atmospheric %)																	
	Pre-Heat Treatment Glucose Levels (mg/dL)					Post-Enzymatic Hydrolysis Glucose Levels (mg/dL)						Ethanol Yields (%)						
	Trial 1	Trial 2	Trial 3	Average	Standard Deviation	Trial 1	Trial 2	Trial 3	Average	Standard Deviation	Trial 1	Trial 2	Trial 3	Average	Standard Deviation			
Oxalic Acid	215	280	213	236	38.12	276	355	324	318	39.80	3.28%	1.856%	2.23%	2.46%	0.74%			
Citric Acid	253	210	218	227	22.87	390	442	369	400	37.58	0.768%	2.207%	0.952%	1.309%	0.78%			
Calcium Hydroxide	206	192	211	203	10.41	error 6	error 6	error 6	error 6	N/A	1.154%	0.486%	0.896%	0.845%	0.34%			

Fig. 2. This Data table shows the glucose and ethanol measurements for each of the three pretreatment types, oxalic acid, citric acid, and calcium hydroxide. The averages are displayed in bold and it can be seen that the results are consistent. Although Citric Acid had produced the most glucose overall, it can be seen that oxalic acid had the highest ethanol readings, followed by citric acid, then Calcium Hydroxide.

Procedure

When carrying out this experiment, the first step was to create a solution of the biomass sample and water through mixing the 2 grams of each biomass sample with 25mL of water per test tube. Next, the biomass mixtures were placed in a tub of boiling water for 15 minutes to loosen up the hemicellulose and lignin which are useless byproducts of the biomass process. After 15 minutes, the biomass samples were taken out and the samples selected for acid pretreatment were treated with 2 grams of citric acid, oxalic acid and calcium hydroxide and left aside for 30 minutes and later washed. This will ensure fewer inhibitor formation in the treatment samples. 2 grams of the cellulase enzyme was added to treated and control biomass samples and left in the common water bath set at 50 degrees Celsius for 12 hours. The glucose levels were measured in all the samples before and after enzyme treatment. This was followed by adding 2 grams of standard baker’s yeast to all the samples and the samples were once again left in the common water bath set at 33 degree Celsius for a 12-hour period. The test tube caps were unscrewed slightly to prevent gas build-up inside the test tubes. Finally, ethanol levels produced by fermentation were measured using an ethanol probe.

Results

In terms of ethanol measurement, corn husk with Oxalic acid pretreatment produced the most ethanol with 2.46% of solution followed by corn husk with no pretreatment with 2.450%. Next came the woodchips without chemical pretreatment, that produced 2.09% ethanol yield. Following woodchips was grass without chemical pretreatment with ethanol levels of 1.797%. This was followed by corn husk with Citric acid pretreatment

(1.31%) and lastly, corn husk with Calcium Hydroxide pretreatment (0.845%).

In terms of glucose levels, the highest producer was grass with 443mg/dL followed by corn husk-control with 441mg/dL then, corn husk treated with citric acid with 400mg/dL. Second to last, was the woodchips with 386mg/dL and last was the oxalic acid treated corn husk with 338mg/dL. Calcium Hydroxide treated sample displayed a consistent error message due to its incapability to measure in concentrated basic or acidic conditions. Oxalic Acid had the most consistent results with a mean standard deviation of 0.33%.

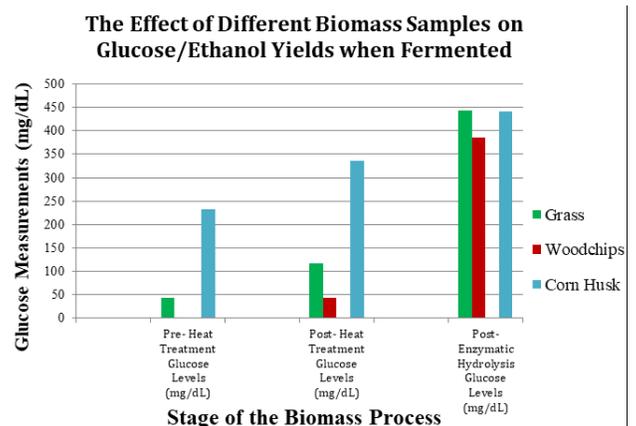


Fig. 3. This graph compares the averages of the glucose levels for each biomass sample. Measurements were taken at three different stages in the biomass process. As seen in the graph, corn husk (purple) had produced the highest glucose yields at the pre- heat treatment phase of the process and again at the post- heat pretreatment stage, followed by grass. In the first stage, woodchips are not displayed due to the errors in reading glucose due to non- blood like conditions.

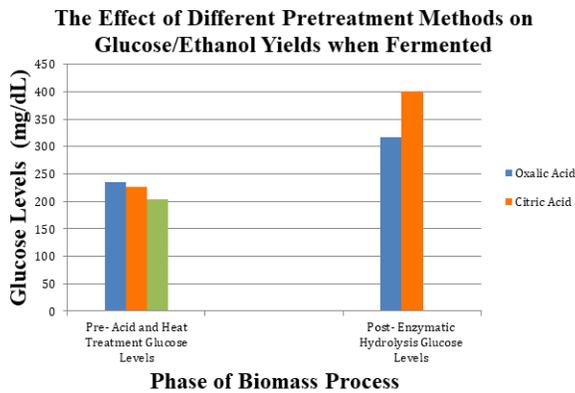


Fig. 4. This graph displays the averages for each of the acid tested biomass samples during the given phases of the process. As shown by the graph, oxalic acid had the highest initial glucose yields followed by citric acid but, in the post-enzymatic hydrolysis phase, citric acid had nearly 50mg/dL more glucose on average than oxalic acid.

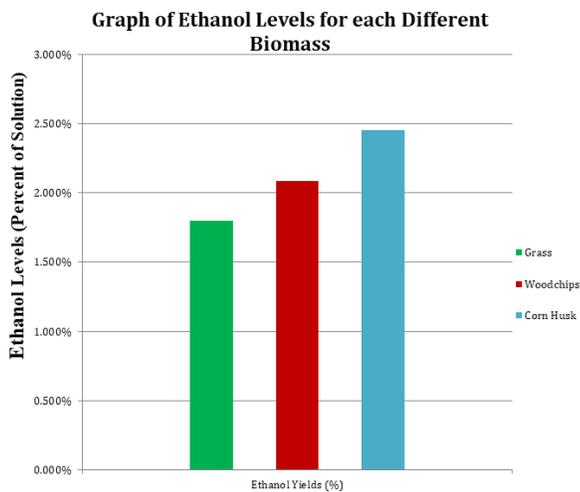


Fig. 5. This graph displays the amount of ethanol produced from each of the three biomass samples when fermented. As seen in the graph, out of the three biomass samples, corn husk produced the highest ethanol yields followed by woodchips and then grass. It can be concluded that corn husk is the ideal biomass type for maximizing ethanol yields.

The Effect of Different Pretreatment Methods on Glucose/Ethanol Yields when Fermented

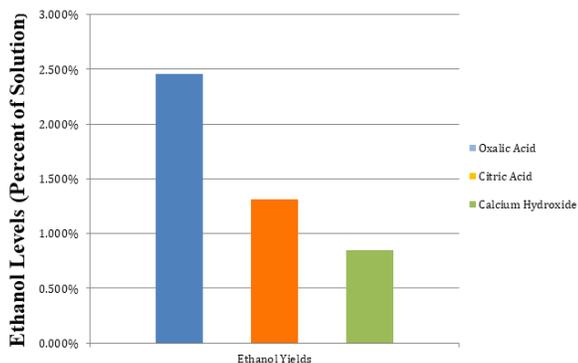


Fig. 6. This graph displays the ethanol levels for each of the acid/alkali treated biomass. As seen by the graph, oxalic acid had produced the highest ethanol yields with 2.46% followed by citric acid, and then calcium hydroxide. Ethanol levels were measured as percent of solution.

Discussion

Data collected from averaging the raw data showed that corn husk produced the highest ethanol levels with a 2.45% average and was the second highest glucose producer, with 441 mg/dL after enzymatic hydrolysis (2mg/dL below the highest, woodchips). Woodchips had the third highest glucose with 386mg/dL and 2.086% ethanol. Grass had the highest glucose levels and lowest ethanol levels. Grass had the most consistent glucose level recordings and most consistent ethanol readings. Woodchips had the highest rate of increase in glucose recordings but had the most inconsistent results.

Oxalic Acid had produced the highest amount of ethanol with 2.46% and second highest glucose measurements. Citric Acid had the second highest measurements of 1.309% ethanol and 400mg/dL of glucose. Calcium Hydroxide produced 0.845% ethanol and had difficulty with glucose readings due to non-blood like conditions. Oxalic Acid had the most consistent data trends. Calcium Hydroxide had the most error messages due to issues with neutralization for more accurate testing but, it had the most consistent results.

These results supported our initial research which showed that organic acids, such as oxalic acid has a more efficient and safe form of biodegradability than other acids and alkali. Because of this, a higher overall ethanol production was to be expected for most of the trials.

This was accurately reflected by both the standard deviation and data collected from the trials.

Future Research could focus on how subjecting the biomass to various levels of pressure in the pretreatment phase will affect the ethanol yield. Another interesting topic to research would be how variations in temperature would affect the degradation speed of the cell wall. This research is important as it can lead to optimal environmental conditions required for extraction of the most plant sugars in the biomass process.

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