Bioethanol from Rice husks as a Second-Generation Biofuel: Glucose Quantification Using Dinitrosalicylic Acid Analysis

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Abstract:

The preferred usage of fossil fuels over renewable energy sources has resulted in the extraneous release of greenhouse gasses into the atmosphere. Greenhouse gasses pollute the atmosphere and contribute significantly to the problem of global warming. As a result, alternative, renewable energy sources have become a central topic for discussion. Biomass is one of many alternatives. Biomass is a more environmentally friendly, renewable organic matter that can be used as fuel. Biofuels that use foods high in carbohydrates, including rice, bread, potatoes, and other crops, are often referred to as first-generation biofuels. However, the problem with first-generation biofuels is that they take away a food source and increase global food prices. Therefore, research has turned to second-generation biofuels, which acquire ethanol from biomass as an alternative to first-generation biofuels. Second-generation biofuels are made from lignocellulose which composes the inedible part of a plant's cell wall composed of cellulose and lignin. This project centralizes utilizing one of the most abundant and readily available biomasses, rice husks. The main objective of this research project is to determine if rice husks are an efficient biofuel. This is determined by converting the rice husk into biofuel using the ionic liquid, known as 1-Butyl-3-methylimidazolium chloride, and quantifying the amount of glucose obtained from this process through the use of dinitrosalicylic acid analysis (DNS), glucose refractometry, and ultraviolet-visible spectroscopy. The greater the amount of glucose in the samples, the more ethanol that can be produced via fermentation to be used as fuel.

Introduction :

Second-generation biofuels comprise lignocellulose, the inedible part of the plant's cell wall that consists of lignin, cellulose, and hemicellulose. Hemicellulose is a polysaccharide found in plant cell walls. Their primary purpose revolves around strengthening the cell wall by interacting with cellulose and, in some cases, lignin. Cellulose is an insoluble substance that contains many chains of glucose monomers and is the main component in plant cell walls and vegetable fibers. Cellulosic biomass is the world's most abundant biological energy source. Lignin is the general term for a group of aromatic polymers found primarily in the walls of secondarily thickened cells. Lignin is a complex and heterogeneous mixture of polymers. Research into biomass conversion to liquid fuel has proven to be quite strenuous. Cellulose and hemicellulose have the potential to be converted into sugars such as glucose and xylose and then fermented into ethanol. The issue is that lignin is a barrier to cellulose and hemicellulose. Therefore, the goal is to break down the lignin to obtain cellulose and hemicellulose to convert it into glucose and xylose. This can be accomplished by using ionic liquids in the pretreatment of biomass. Ionic liquids break biomass into lignin, cellulose, and hemicellulose. Ionic liquids are salts in which ions are not coordinated well, which results in them being liquids below a specific temperature. Ionic liquids can be helpful in synthesis, catalysis, batteries, and fuel cells. The use of ionic liquids in place of more traditional organic solvents is beneficial because it prevents the emission of volatile organic compounds, which is a significant source of environmental pollution. Ionic liquids are also helpful because they can help maximize yield, selectivity, substrate solubility, product separation, and even enantioselectivity.

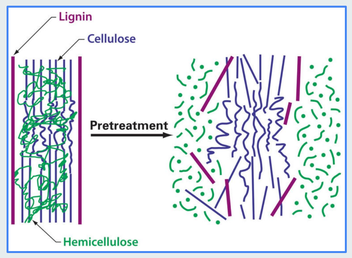

Figure 1. Visual representation of the pretreatment breaking down lignocellulose into hemicellulose, cellulose, and lignin.

Procedure:

I. Ionic Liquid(IL) Pretreatment and Acid Hydrolysis

The Ionic Liquid pretreatment involves 1-Butyl-3-methylimidazolium chloride. 1.5 grams of 1-Butyl-3-methylimidazolium chloride was weighed out using an electronic top-loading balance and placed in a 25 mL flask accompanying 0.3 grams of ground up rice husks. The ratio of Ionic Liquid to rice husk was 1:5 for each sample. The samples were placed in dishes filled with mineral oil and heated to 80℃ for 3, 6 or 9 hours. Each sample received 10.0 mL of 0.5 M Hydrochloric acid and a magnetic stirrer. The different samples were placed in dishes filled with mineral oil and heated to 80 degrees Celsius for 3, 6 or 9 hours. After each sample was removed from the mineral oil and cooled, 10.0 mL of 0.5 M sodium hydroxide solution was poured into each sample.

Figure 2. The samples were heated in a mineral oil bath for three, six, or nine hours at 80°C. A magnetic stirrer is added to mix the solution.

II. Centrifugation

After each sample was cooled to room temperature, the samples were placed in a centrifuge for 10 minutes and spun at 2010 rpm. Then they were spun a second time for 5 minutes at 2500 rpm. Each sample was placed in a slot opposite of another in order for there to be a balance of masses.

III. Filtration

The filtration setup involved taking glass pipettes and breaking them at the tip of the pipette. Each pipette was filled with glass wool fiber, sand, and charcoal. The glass wool fiber was inserted into the pipette first, followed by sand, charcoal. The charcoal filters out the color of the sample from brown to clear. The liquid from the samples are pipetted into each filter. Samples are filtered until clear.

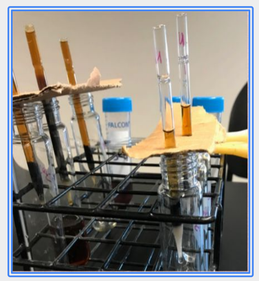

Figure 3. Centrifuged samples were placed in a filtration system consisting of glass fiber, sand, and charcoal in a short-necked pipette. The solutions were filtered until they were clear.

IV. DNS Analysis

After obtaining clear solutions, a DNS reagent was made. 7.5 g of potassium sodium tartrate (Rochelle salt), 20 mL of 2.0M sodium hydroxide, 0.0125g of sodium sulfite, 0.25 g of dinitrosalicylic acid (DNS), and 250 mL of distilled water were added to create the DNS reagent. Seven samples containing DNS reagent and varying glucose concentrations were analyzed to obtain a standard curve. The samples were then heated in boiling water for 12 minutes and transferred to an ice bath for 15 seconds.

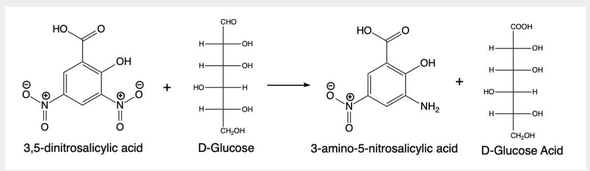

Figure 4. Structural representation of the DNS reaction scheme.

Results:

Figure 5. Experimental standard curve from DNS analysis.

As the concentration of glucose increases, a color change from yellow to an amber brown color is observed (Figure 5). From the experimental standard curve, all of the samples changed color and switched from bright yellow to amber with the varying concentrations of glucose. Absorbances were taken of the data and plotted as a function of the known glucose concentration (Figure 6).

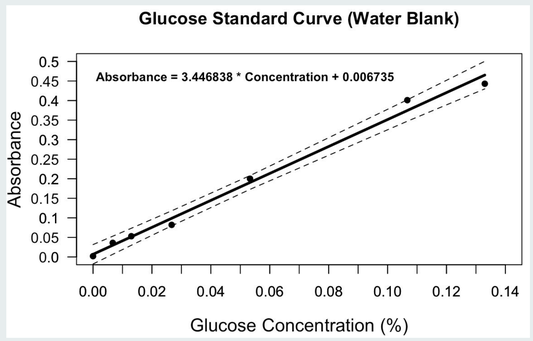

Figure 6. Graphical representation of our standard curve.

We found that the absorbance of our solutions increased as the concentration of glucose also increased ($F = 598$; $df=1, 5$; $p < 0.001$; $R^2 = 0.99$; Absorbance water = 3.446838*Concentration + 0.006735).

Sample	Absorbance	Solved Concentration w/o error	Absolute Error(+/-)	Refractometer Data	Refractometer Margin of Error	Absolute Error
3,3	2.402	0.694916782	0.029255997	0.665	0.01	0.00665
3,6	2.45	0.708842591	0.029842273	0.65	0.01	0.0065
3,9	2.493	0.721317794	0.030367479	0.693	0.01	0.00693
6,3	1.974	0.570744987	0.024028364	0.57	0.01	0.0057
6,6	1.731	0.500245581	0.021060339	0.28	0.01	0.0028
6,9	N/A	#VALUE!	#VALUE!	N/A	0.01	#VALUE!
9,3	2.396	0.693176056	0.029182712	0.689	0.01	0.00689
9,6	1.774	0.512720784	0.021585545	0.32	0.01	0.0032
9,9	1.832	0.529547803	0.022293963	0.359	0.01	0.00359

Figure 7. Table for the collected data of our first sample run

Future Directions

Future directions include quantifying the amount of glucose in each of our samples, via a glucose refractometer and a graphical interpolation followed by analysis for agreement. Following this, the heating time and conditions can be compared and analyzed via a one way ANOVA to determine which treatment would produce a solution with the most active glucose concentrations.

References and Acknowledgements:

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