# Bioethanol Production: Glucose Testing and Quantification Using DNS Analysis and Addition Analytical Methods

Dineen Vogler and Barnabas Gikonyo, Chemistry Department, SUNY Geneseo

## Introduction

The search for alternative ways to create a more sustainable and environmentally friendly source of energy, other than fossil fuels, has been ongoing for many years. Particularly, the production of biofuels has become of interest. One effort has been through the creation of first-generation biofuels that curb greenhouse gas emissions, unlike fossil fuels. However, first generation biofuels lead to the increase in food prices which negatively impacts developing countries, as they use food sources to produce biofuel. To mitigate this issue, second generation biofuels are considered as a better alternative. Instead of hindering the food supply, second generation biofuels aim to not affect the food supply, as they use non-human food sources. Furthermore, second generation biofuels are relatively inexpensive.

Second-generation biofuels are made from lignocellulose, which is the inedible part of the plant's cell wall that is made up of lignin and cellulose. Cellulose and hemicellulose have the power to be converted into sugars such as glucose and xylose which is then fermented into ethanol. Ionic liquids are used in the pretreatment of biomass. Ionic liquids are essential towards aiding the breakdown of the biomass into cellulose, lignin and hemicellulose. The goal is to get the cellulose so that it can be converted into glucose and to eliminate lignin while retaining the hemicellulose.

In order to determine the effectiveness of biofuels derived from rice husks, glucose quantification is a necessary step. Two methods that allow for the concentration of glucose to be quantified are dinitrosalicylic acid (DNS) analysis and the standard addition method. Through the analysis of each method, it can be determined if DNS analysis and the standard addition method are reliable glucose quantification procedures.

## Methodology

### A) Preparation of Rice husks

Prior to glucose quantification, rice husks are washed with deionized water, chopped and dried in an oven at 70 degrees Celsius. Dried rice husks were then ground up, using a mortar and pestle and distributed among nine samples, each containing 0.3 grams of ground up rice husk. Next, the rice husks underwent an ionic liquid pretreatment using 1-butyl-3-methylimidazium chloride. Samples are then placed in dishes with mineral oil and heated to 80 degrees Celsius for 3,6, or 9 hours. Then, 0.5 M Hydrochloric acid is added, and samples went back in the mineral oil for 3,6, or 9 hours. After samples are cool, 0.5 M sodium hydroxide is added to stop the reaction and then subjected to centrifugation. Samples are then filtered using a short-necked pipette with glass fibers, sand, and charcoal inside. Filtration stops when a clear solution is obtained.

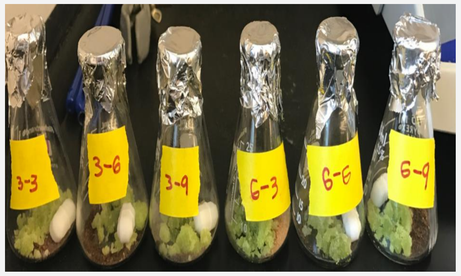



***Figure 1.***
Samples labeling: the first being the amount of time that the sample spent after subjection to the ionic liquid and the second number indicates the time spent in the mineral oil bath after treatment with 0.5 M hydrochloric acid.


***Figure 2.***
Samples are heated in a mineral oil bath for 3,6, or 9 hours at 80 degrees Celsius. A magnetic stirrer is added to completely mix the mixture

***Figure 3.***
Centrifuged samples are subjected to a filtration system consisting of glass fibers, sand, and charcoal in a short-necked pipette. Solutions are filtered until clear.

### B) DNS (dinitrosalicylic acid) analysis

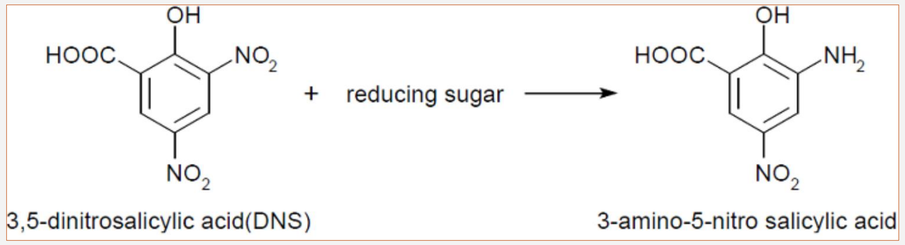
The suggested composition of the DNS reagent consists of dinitrosalicylic acid, Rochelle salt, sodium hydroxide, and sodium bisulfite. In previous experiments, impurities brought by the presence of phenol in the DNS reagent contributed to the overestimation of total reducing sugars. It should be mentioned that sodium bisulfite is added prior to the use of the reagent, to prevent atmospheric oxidation.

### C) Standard Addition Method

Standard addition method is a common analytical method to determine the concentration of an unknown analyte. One way that this method can be carried out is by adding increasing amounts of known quantities of analyte to samples with equal volumes of solution of interest. From there, samples are diluted with water to obtain equal volumes. To determine the initial concentration of an analyte, a calibration curve can be made.

## *Glucose Testing and Quantification Methods*

### 1. DNS (dinitrosalicylic acid) analysis:

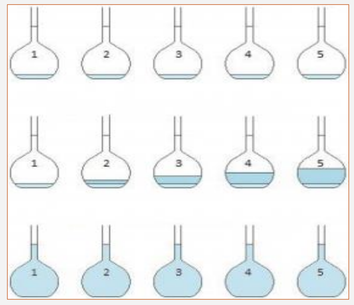



Picture from: iGEM Kyoto

***Figure 4.***
With the presence of heat, the reducing sugar donates a hydrogen atom to DNS by losing a hydrogen atom itself. Therefore, the reducing sugar is oxidized and DNS is reduced. When this redox reaction occurs, a color change from *yellow to orange/red* is observed. From there, the absorbance can be measured with a spectrophotometer to determine the initial concentration of glucose, the reducing sugar.

### 2. Standard Addition Method (SAM):

***Figure 5.***
The standard addition method is used to determine the initial concentration of a particular analyte. And simultaneously removes matrix effects. This method assumes that one analyte has one specific signal that is recognized by an analytical sensor. The procedure implemented is visualized in the following picture. Note that only rotational effects can be corrected for, meaning that only the slope is impacted.




Picture from: JMP Statistical Discovery from SAS

### 3. Generalized standard addition method (GSAM):

- Considers interference effects and quantifies reducing sugars simultaneously
- Multiple standard additions are required
- Multiple regression model is created

## Expected Results

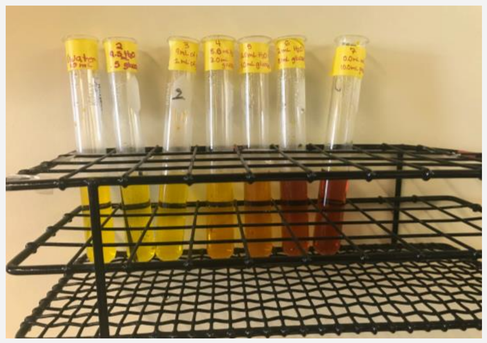
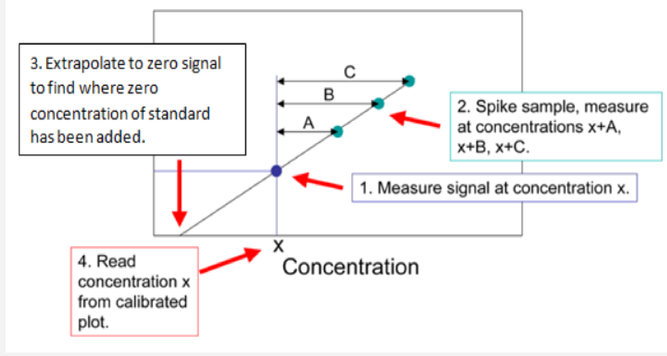


***Figure 6.***
A standard curve for the DNS analysis has been made. As the volume of glucose increases and the volume of water decreases, the color of the solution transitions from yellow to orange/red. The absorbance of the samples can be measured through spectrometry.




Picture from: Chemistry Libre Texts

***Figure 7.***
A linear regression model is made to determine the initial concentration of glucose. Through the extrapolation of the line, the x intercept can be obtained. The x intercept value corresponds to the initial concentration of glucose in the sample.

## Conclusion & Future Directions

The limitations brought upon by DNS analysis and addition analytical methods suggest that there is not one method that is 100% reliable. Therefore, more than one glucose quantification tests should be performed to maximize accuracy in determining the concentration of glucose in the rice husk samples. Future directions for the glucose testing and quantification would include the implementation of other tests for reducing sugars: Fehling's Test or Tollen's test. The effectiveness of these tests could be compared to the ability of the DNS method and Benedict's Test.

## Literature Cited

1. Abbas, A., & Ansumali, S. (2010). Global potential of rice husk as a renewable feedstock for ethanol biofuel production. Bioenergy Research, 3(4), 328-334.
2. Deshavath, N. N., Mukherjee, G., Goud, V. V., Veeranki, V. D., & Sastri, C. V. (2020). Pitfalls in the 3, 5-dinitrosalicylic acid (DNS) assay for the reducing sugars: Interference of furfural and 5-hydroxymethylfurfural. International Journal of Biological Macromolecules, 156, 180–185. https://doi.org/10.1016/j.ijbiomac.2020.04.045
3. Ellison, S. L. R., & Thompson, M. (2008). Standard additions: Myth and reality. The Analyst, 133(8), 992. https://doi.org/10.1039/b717660k
4. Lee, L. P., N. H. Hassan, and M. R. Yusop. "1-Butyl-3-Methylimidazolium Chloride Pretreatment on Malaysia Lignocellulosic Wastes." Malaysian Journal of Analytical Sciences, vol. 19, no. 1, 2015, pp. 20-30, SCOPUS.
5. Miller, G. L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry, 31(3), 426–428. https://doi.org/10.1021/ac60147a030
6. Naik, S. N., Goud, V.V., Rout, P.K., Dalai, A. K. (2010). Production of first and second generation biofuels: A comprehensive review. Renewable and Sustainable Energy Reviews, 14(2), 578-597.
7. Saxberg, Bo E. H., B.R. Kowalski. "Generalized Standard Addition Method." Analytical Chemistry, vole 51, no. 7, 1979
8. Scheller HV, Ulvskov P. Hemicelluloses. Annu Rev Plant Biol. 2010;61:263-89.

## Acknowledgements

The author graciously acknowledge financial support from the Office of Sponsored Research and the McNair Program, as well as material and logistical support from the Chemistry Department.