8-2018

Hydrolysis of Cellulose and Biomass Using Blue Molybdenum

Farouk Awudu
East Tennessee State University

Hydrolysis of Cellulose and Biomass Using Blue Molybdenum

A thesis

presented to

the faculty of the Department of Chemistry

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Master of Science in Chemistry

---

by

Farouk Awudu

August 2018

Dr. Dane W. Scott

Dr. Robert Standaert

Dr. Aleksey N. Vasiliev

Keywords: Hydrolysis, Cellulose, Biomass, Blue molybdenum, Reducible Sugars

ABSTRACT

Hydrolysis of Cellulose and Biomass Using Blue Molybdenum

by

Farouk Awudu

Hydrolysis of cellulosic biomass is an important and ongoing subject of research due to generating precursors for biofuel synthesis. This work involves hydrolysis of microcrystalline cellulose and Arundo donax to glucose. Methods for acid hydrolysis include the use of sulfuric acid, phosphomolybdic acid, blue molybdenum using low concentrations at 40-100 °C. For comparison purposes, enzymatic hydrolysis was also carried out using cellulase. Products were quantified by measuring total organic carbon and reducible sugars using dinitrosalicylic acid. Use of phosphomolybdic acid, blue molybdenum and sulfuric acid even at 6.0 M did not increase the amount of glucose compared to using water only. Interestingly, enzymatic hydrolysis of powdered Arundo donax without pretreatment was successful and resulted in statistically similar amounts of glucose compared to using microcrystalline cellulose. Efforts are ongoing to understand the enzyme kinetics in the hydrolysis of Arundo donax and potentially increase the yield of glucose using chemical and microbiological pretreatment.

DEDICATION

This work is dedicated to my mother: Hajia Hajara Mahmud. My brothers: Abdul Aziz Bamba, Abdul Aziz Safianu and Abdul Aziz Binyamin. My sisters: Mrs. Mariam Mahama, and Abdul Aziz Sahadatu. My son: Abdul Aziz Farouk. My friends: Ben Ahiadu, Joel Annor-Gyamfi and Duke Debrah Asante.

ACKNOWLEDGEMENTS

First and foremost, I thank God for the successful completion of this work and my academic pursuit at the East Tennessee State University.

I would like to thank my advisor, Dr. Dane W. Scott for his patience, guidance and mentorship during my research. I would like to express my profound gratitude to Dr. Bob Standaert and Dr. Aleksay Vasiliev for serving as my thesis advisory committee members.

Lastly, my sincere appreciation goes to all my colleagues and professors in the Chemistry Department of East Tennessee State University.

TABLE OF CONTENTS

Page

LIST OF TABLES

TABLE OF FIGURES

LIST OF ABBREVIATIONS

TOC	Total organic carbon
DNS	Dinitrosalicylic acid
PMA	Phosphomolybdic acid
BM	Blue molybdenum
n	Degree of polymerization
KHP	Potassium hydrogen phthalate
^{o}C	Degree Celsius
ppm	Parts per million
ppt	Parts per thousand
g	gram
mol	mole
T	Temperature
M	Molarity

CHAPTER 1

INTRODUCTION

Cellulose and Biomass

Conversion of cellulose to glucose has attracted significant attention due to the potential of resulting in a sustainable source of renewable energy [1,2]. The production of biofuel from biomass as an alternative source of energy will significantly address environmental pollution and the greenhouse effect emanating from mainly carbon dioxide emission leading to global warming. This has the potential to bring about foreign exchange savings as a result of reduced dependence on fossil fuels and energy security. In addition, it will create a market for the agricultural sector through the supply of biofuel feedstocks to boost the economy of both industrialized and developing countries [3].

Cellulose is the main component in plants referred to as biomass [1,4]. There is approximately 1.5×10^{12} tons of the total biomass produced annually [4,6.] As such, there is significant interest in converting biomass to glucose. Cellulose is a linear polysaccharide with D-glucose monomeric units connected by β (1→4) glycosidic bonds which are formed through hydrogen bonding. However, the strong hydrogen network in the cellulose polymer results in the formation of a robust crystalline structure shown in Figure 1. This results in cellulose being insoluble in water [5].

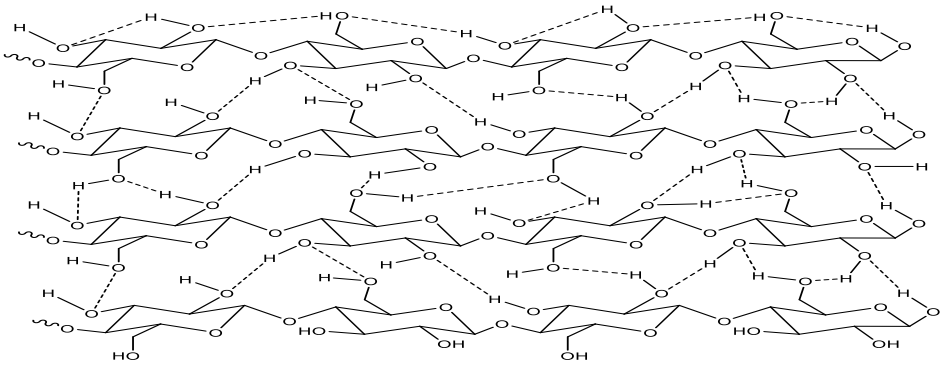

Figure 1. Crystalline structure of cellulose [2]

Cellulose from different sources have different chain lengths. The chain length of cellulose is expressed in terms of degree of polymerization, n, shown in Figure 2. Wood pulps and cotton are the main sources of cellulose for industrial use [6]. The degree of polymerization of wood pulps ranges between 500 to 2,100 and that of cotton ranges between 500 to 3,000 [7,8]. Microcrystalline cellulose is produced by incomplete depolymerization of cellulose substrate resulting in a degree of polymerization from 150 to 300 [6].

Although hemicellulose and lignin are also components of lignocellulose, cellulose is the major constituent with a percentage composition of 40% to 50% [8].

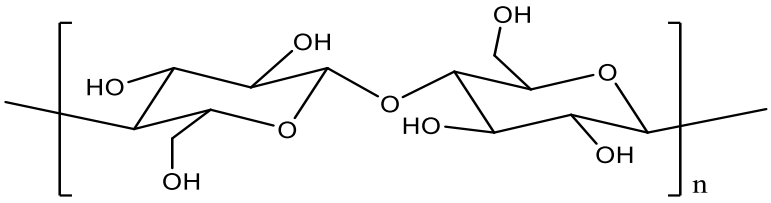

Figure 2. Example of cellulose polymer in which n ranges between 400-1,000 [9]

These different components of cellulose makes it possible to explore its chemistry due to its sensitivity towards hydrolysis and oxidation [6]. Cellulose is also a raw material to produce environmentally friendly products such as paper, rayon, and cellophane [10]. Glucose produced from cellulose can further be converted to ethanol which can serve as a biofuel [11].

Other biofuels through gasification, pyrolysis and hydrolysis are a result of chemical processes. Syngas is produced by gasification and can further be converted to methanol while pyrolysis and hydrolysis of cellulose leads to the production of biofuel precursors such as glucose [8]. Biofuels can also be produced from food products like corn and waste vegetable oil. The use of food products as renewable source of energy is not economically sustainable since it might lead to a shortage and high cost of food products [3]. Cellulose from non-food based products are a better source due to low-cost and abundance [12]. One example is the plant arundo donax.

Arundo Donax

Arundo donax is a perennial crop commonly referred to as giant cane or giant reed. It belongs to the family of Poaceae Arundinaceae. It is an energy crop which has a high biomass yield per hectare [13,14]. The herbaceous plant is common along water bodies such as ponds, rivers and marshes. The unique feature of this plant is its ability to adapt to different kinds of soil which makes it a readily adaptable source of biomass. The cultivation of this plant is less expensive compared to other energy crops since it is less labor-intensive and also requires low agronomic inputs [15]. Dried Arundo donax is a biomass classified as an energy crop and is a better alternative compared to other agricultural commodities such as wheat and corn as a biofuel feedstock [13].

Dissolution of Cellulose

Extensive research has been conducted in cellulose degradation chemistry [3,6]. Dissolution of cellulose biopolymer enhances hydrolysis and this is achieved by several pretreatment methods using acidic solutions, treatment of the cellulose substrate with lime, and alkaline solutions. Supercritical water and enzymes have also been used to weaken the hydrogen bond network in cellulose to increase its solubility prior to hydrolysis [16].

Although the afore-mentioned pretreatment methods enhance the solubility of cellulose, they come with disadvantages including difficulty in separation of cellulose products from the catalyst, corrosion hazards and redundant neutralization products [16]. In addition, some solvents require strict temperature control and composition in order to enhance the solubility of cellulose. These pretreatment methods tend to hamper the economic viability of these alternative routes due to high cost of production [17].

There are two classes of cellulose solvents namely, derivative and non-derivative aqueous solvent systems listed in Table 1. In derivative solvent systems, there is a chemical interaction between the cellulose and the solvent which results in the formation of cellulose derivatives. The cellulose derivatives have the ability to decompose to regenerate the cellulose biopolymer [16,18]. In non-derivative solvent systems, cellulose dissolves by intermolecular interactions only. This type of solvent is commonly used for dissolving cellulose and preparing other products from cellulose [16,18].

Table 1. Examples of derivative and non-derivative solvents [16,18]

Aqueous Derivative Solvent Systems	**Aqueous Non-Derivative Solvent Systems**
Zinc Chloride	Lithium Chloride
Trifluoroacetic Anydride	N-dimethyl acetamide
Trifluoroacetic Acid	Ammonia/Ammonium Thiocyanate
Carbon Disulfide	Hydrazine/Thiocyanate
Formic Acid	Sodium Hydroxide/Urea
Pyridine	Sodium Hydroxide/Thiourea
	Lithium Hydroxide/Urea

Although not all of these aqueous solvent systems have the ability to dissolve cellulose at room temperature, sodium hydroxide/urea, sodium hydroxide/thiourea and lithium hydroxide/urea dissolve cellulose. Dissolution occurs under strict conditions of temperature, the molecular weight of cellulose and concentration of solvent [1,16,19].

Hydrolysis of Cellulose

Hydrolysis of cellulose involves the cleavage of the β (1→4) linkages between the glucose units. However, the rigidity and crystalline nature of cellulose due to the strong inter-molecular and intra-molecular hydrogen bonds make it difficult for solvents to penetrate the biopolymer to break the β (1→4) linkage. This reduces the efficiency of hydrolysis leading to low yield of glucose and hydrolysis products [17,20].

Enzymatic Hydrolysis of Cellulose

Conversion of cellulose to glucose can also be achieved using enzymes. *Trichoderma Reesei* is a type of filamentus fungus enzyme that excretes cellulosic enzymes such as cellulase. These enzymes are composed of endo-p-glucanase, exo-p-glucanase, and glucosidase, which are all required for hydrolysis of cellulose [7].

Hydrolysis involves two main steps shown in Figure 3. First, the cellulose is hydrolyzed into cellobiose by the synergistic effects of endo-p-glucanase and exo-glucanase. The second step is conversion into glucose by beta-glucosidase as shown in Figure 3 [7].

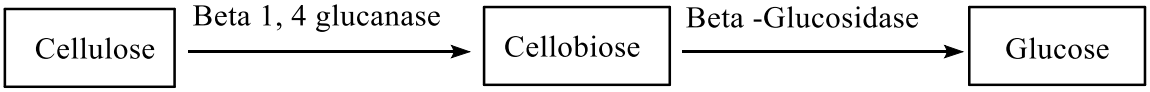

Figure 3. Flowchart of enzymatic hydrolysis of cellulose to glucose [7]

Although enzymes can be used to convert cellulose to glucose, specific aqueous solvents and mild reaction conditions are required to promote hydrolysis of cellulose. This process is not feasible on a commercial scale because of cost.[1]

Acid Hydrolysis of Cellulose

Acid hydrolysis can be used in the degradation of cellulose into soluble products such as glucose, furfurals, and levulinic acid and involve steps shown in Figure 4 [7]. Acid hydrolysis can be carried out under dilute or concentrated conditions. Dilute hydrolysis requires relatively high temperature and pressure which increases the rate of degradation of cellulose [7]. Hydrolysis of pure cellulose using 1% sulfuric acid at a temperature of 237 ˚C produced over 50% yield of sugars [7]. Due to the high temperature required in this process, special reactor materials are used which makes this process expensive [7, 21].

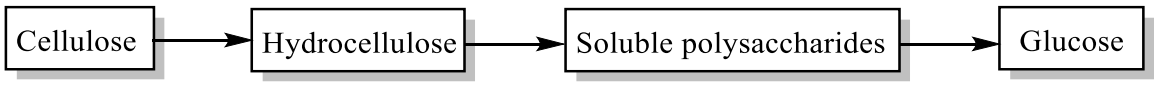

Figure 4. Flowchart of dilute acid hydrolysis [6,7]

Concentrated acid hydrolysis on the other hand uses relatively mild temperatures and low pressures. The steps forming glucose is shown in Figure 5. This chemical process gives a higher yield of sugars than dilute acid hydrolysis and it is cheaper because no special reactor materials are required. However, the reaction is slow, expensive, and difficult to recover and separate the acid used since it requires a large amount of base to neutralize the acid [7].

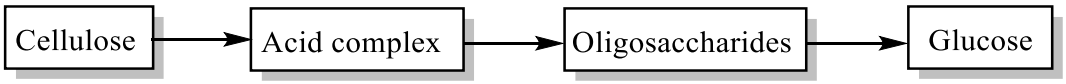

Figure 5. Flowchart of concentrated acid hydrolysis [7]

Research conducted by Saeman showed that the maximum yield of sugar changes with varying reaction conditions such as temperature and concentration of acid used in the hydrolysis. Under these conditions, other hydrolysis products are also possible including decomposition of glucose. Increasing the temperature increases the rate of cellulose hydrolysis more significantly than the rate of decomposition of glucose [22,23].

The mechanism of acid hydrolysis of cellulose is given in Figure 6. Depolymerization of cellulose by acid-catalyzed hydrolysis involves the protonation of the oxygen in the β (1→4) glycosidic bond. This leads to the subsequent cleavage of the glycosidic bond in water yielding glucose monomers which can be used as a biofuel precursor [24].

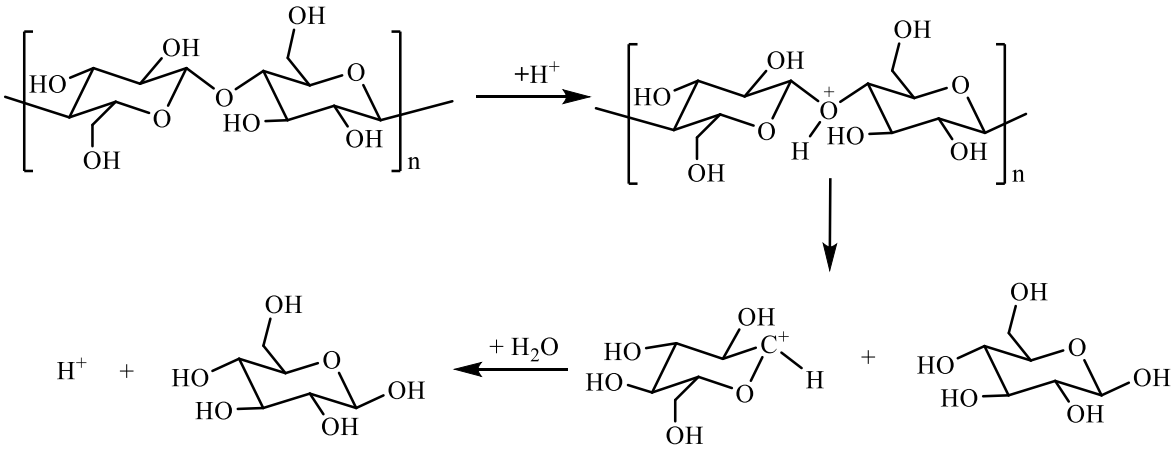

Figure 6. Mechanism of acid-catalyzed hydrolysis of cellulose to glucose [7,24]

Some examples of catalysts used in the conversion of cellulose to glucose and other derivatives at different temperatures include nafion/iron (III) chloride supported on amorphous silica.[1] Other examples include 1-butyl sulfonic acid and 3-methylimidazolium hydrogen sulfate, [5] zeolites or sulfated zirconia supported on mesoporous silica [25].

Phosphomolybdic Acid and Zinc

Extensive research into cellulose chemistry has been conducted using heteropoly acids (HPAs) as catalysts in the chemical transformation of cellulose to biocompatible products [26,27]. The high acidity and solubility of HPAs in water enhance the efficiency of cellulose catalyzed reactions.[28] HPAs are also efficient in catalysis at both low concentrations and temperatures due to their high catalytic property [13]. They also have the potential to convert raw biomass like hay to glycolic acid [28].

Phosphomlolybdic acid (PMA) is a Keggin-type HPA which has a general formula of $H_3PMo_{12}O_{40}$. It has attracted significant attention because it is inexpensive and environmentally

friendly. It is a solid acid catalyst which has low vapor-pressure compared to volatile liquid acids such as sulfuric acid and hydrochloric acid [28]. It also has the potential to be recycled in the production of c-glycosides, O- and S-glycopyranosides [12]. Additionally, PMA is a yellow-green complex and the addition of a reducing agent, such as zinc, reduces the molybdenum resulting in blue molybdenum (BM) which has catalytic properties [29]. The reduction reaction is shown in equation (1).The significant abundance and low cost of zinc compared to other metal catalysts such as Pd, Rh, Ru and Ir make this route of preparing blue molybdenum ideal [30].

$$H_3PMo(VI)_{12}O_{40} + 2e^- \rightarrow [H_3PMo(VI)_{12-n}Mo(V)_n]^{n-} \quad (1)$$

PMA (Yellow) BM (Blue)

Research Objective

This work aimed to easily quantify glucose, which is a biofuel precursor, using reagents that are both cost-effective and environmentally friendly. Specifically, hydrolysis of Arundo donax is of interest due to being an excellent renewable source of cellulose. To meet this objective, hydrolysis was carried out using water as a blank, sulfuric acid, phosphomolybdic acid, blue molybdenum (BM) and cellulase. Blue molybdenum is easily prepared by dissolving phosphomolybdic acid in deionized water and adding zinc to reduce the molybdenum and generate hydrogen which is incorporated into the oxide matrix turning the solution blue resulting in the term blue molybdenum. Interestingly, the pH of 0.001 M blue molybdenum is identical to 0.001 M sulfuric acid meaning this reagent is amenable to carrying out acid hydrolysis under dilute acidic conditions. Different temperatures were used to determine the temperature dependence of hydrolysis. Enzymatic hydrolysis was carried out at 37 °C using cellulase buffered at a pH of 5.0. Trials determining the reaction rate forming glucose over time are

ongoing. The products quantified to determine the effectiveness of BM are total organic carbon (TOC) and glucose using the dinitrosalicylic acid (DNS) assay method. The DNS method results in the amount of glucose as a result of acid hydrolysis. Other reducible sugars result in a positive test using the DNS method and are in the process of being determined.

CHAPTER 2

EXPERIMENTAL METHODS

The flowchart in Figure 7 shows the methods and order of analysis used in this work.

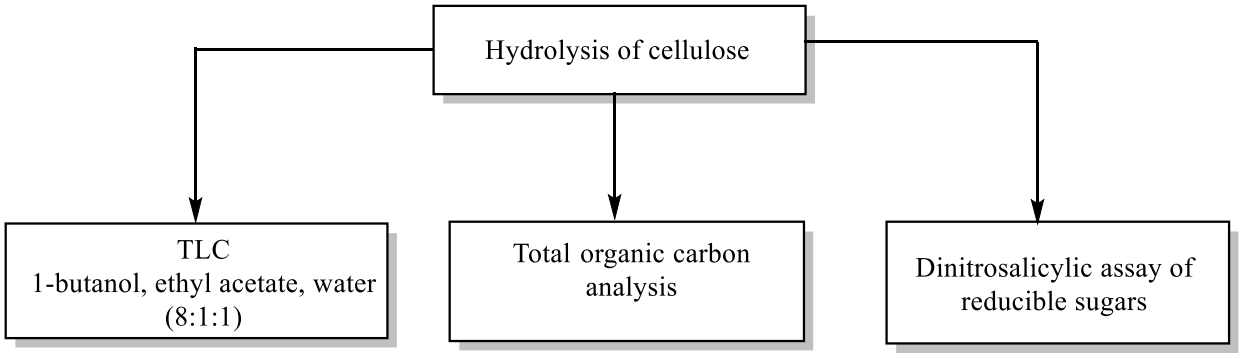

Figure 7. Flowchart of analysis of the cellulose products

Chemicals, Materials and Equipment

Microcrystalline cellulose powder, phosphomolybdic acid ($H_3PMo_{12}O_{40}$), sulfuric acid (H_2SO_4), glucose, cellulase, dinitrosalicylic acid, silver nitrate ($AgNO_3$), 1-butanol, ethyl acetate, sodium hydroxide, acetate buffer , and sodium potassium tartrate were used as received. Pure water (18 MΩ) is generated using a Millipore system. Equipment necessary to carry out the experimental includes Whatman TLC plates 20 cm × 10 cm, TLC capillaries, Hatch DRB 200 Reactor, magnetic stirrer, Vernier® Spetrovis Plus Spectrometer, Vernier pH probe and Logger Pro 3 software. Total organic carbon kits were purchased from Hach (#2760345). These kits include digestion vials, packets of persulfate powder (0.1 g each), indicator thymol blue ampules and buffer (sodium bisulfate). Arundo donax was kindly provided by Dr. Ranney at the North Carolina Cooperative Extension. An inexpensive blender was used for generating a powder form of the Arundo donax.

Calibration of Hatch DRB 200-3 Instrument with KHP

Potassium Hydrogen Phthalate (KHP) solutions of 2.00, 4.00, 6.00, 8.00, 10.00, and 20.00 ppm were prepared and used as primary standards to develop a calibration curve for determining total organic carbon [31]. For each standard, 10 mL was added to 0.4 mL of buffer solution (pH = 2.00) and stirred magnetically for 10 minutes. Three 3.00 mL aliquots were transferred to clean digestion vials each containing 0.1 g of persulfate. The same procedure was used with deionized water without KHP as a blank. A blue indicator ampule was opened and placed in each digestion vial in an upright position and then the vial was sealed to ensure it was air tight. The samples were placed in the DRB 200 reactor preset at 105 °C and heated for 2 hours. The samples were allowed to cool for an hour. The absorbance was measured with a Vernier VIS Spectrovis Plus spectrophotometer using Logger Pro 3 software. A maximum wavelength (λ_{max}) of absorbance was found to be at 591.8 nm. The difference in the absorbance of each sample and the blank was used to generate a calibration curve for total organic carbon.

pH Measurements

The pH of the solutions were measured using a calibrated Vernier pH probe. Three vials were prepared in which one contained 0.1 g of sulfuric acid in 4.00 mL of water 2.0 g of PMA was added to the other two vials also containing 4.00 mL of water. The blue molybdenum solution was obtained by adding 60 mg of zinc to the third vial.

Acid Hydrolysis of Microcrystalline Cellulose

Three digestion vials were prepared by adding 0.1 g cellulose to 4.00 mL of deionized water as a blank. Three additional vials using 0.1 g concentrated sulfuric acid in 4.00 mL of water were set up and 0.1 g of microcrystalline cellulose was added to each vial and sealed. The

samples were placed in a DRB 200 digester, heated for 2 hours at 40 °C, cooled for an hour and filtered to remove the unhydrolyzed cellulose using syringes (VWR International 25 mm) with nylon membrane filters (0.45 µm). The filtrates were each transferred into separate 200.0 mL volumetric flasks and diluted with deionized water. This procedure was repeated at 60 °C, 80 °C, and 100 °C. Two additional vials containing 2.0 g of PMA dissolved in 4 mL of deionized water were prepared. One vial served as a blank using PMA and 60 mg of zinc powder was added to second vial resulting in BM. A total of 6 vials were set up with 2.0 g PMA, 4.00 mL water, 0.1 g microcrystalline cellulose and 60 mg of zinc was added to three of the vials resulting in BM. Acid hydrolysis was carried out at 100 °C. The samples were cooled, filtered and diluted to 200.0 mL.

Acid Hydrolysis of Arundo Donax

Arundo donax plants were cut into small pieces and dried in an oven. The pieces were placed in a blender for 4 hours resulting in a powder. Six vials containing 4.00 mL of water was set up. To three vials 0.1 g sulfuric acid was added. To the remaining three vials 2.0 g of PMA and 60 mg of zinc was added. Powdered Arundo donax, 0.1 g, was added to each vial. A blank sample containing 0.1 g of the powdered stalk of Arundo donax and 4 mL of deionized water was prepared and labeled accordingly. The samples were heated in the DRB 200 digester for 2 hours at a constant temperature of 100 °C. The samples were cooled for 1 hour and then centrifuged to separate the plant residue. The supernatant, 4.00 mL, of each sample was diluted to 200.0 mL in a volumetric flask.

Enzymatic Hydrolysis of Cellulose and Arundo Donax

Cellulase carries out enzymatic hydrolysis of cellulose at a pH of 5.0. This requires experiments with and without cellulase to determine the amount of glucose as a direct result of

enzymatic hydrolysis. Microcrystalline cellulose, 0.5 g, and 100 mL of 0.05 M acetate buffer solution at a pH of 5.0 were heated to 37.0 °C and stirred for 2 hours. The mixture was filtered and diluted to 500.0 mL. This procedure was repeated with the addition of 0.5 g cellulase. Powdered Arundo donax without pretreatment was tested following this procedure.

Thin-Layer Chromatography

A thin-layer chromatography procedure for separating monosaccharides and disaccharides was modified to separate reducible sugars which are known to be present in the hydrolyzed cellulose and Arundo donax samples [32]. Approximately 5 mL of the hydrolyzed cellulose samples using phosphomolybdic acid and blue molybdenum catalysts were neutralized with sodium hydroxide (1.0 M) respectively. Different TLC capillaries were used to spot the neutralized samples and pure glucose solution standard (0.0556M) on 20cm ×10cm Whatman TLC plates accordingly. The spots were eluted using a mixture of 1-butanol, ethyl acetate and water in the ratio of 8:1:1 by volume. The TLC plate was dried at room temperature and then sprayed with an alkaline solution containing silver nitrate and ammonia hydroxide. The TLC plate was dried in an oven for 5 minutes prior to visualization of the compounds separated on the plate. Another TLC experiment was conducted on two separate samples containing a mixture of pure glucose/phosphomolybdic acid and pure glucose/blue molybdenum. This served as a control experiment to compare the effect of the catalysts on the separation of glucose and other reducible sugars that might be present in the hydrolyzed cellulose.

Dinitrosalicylic Acid (DNS) Method

Approximately 1.009 g of dinitrosalicylic acid, 3.011 g of potassium tartrate and 1.627 g of sodium hydroxide was transferred into separate 50 mL Erlenmeyer flasks each containing 20 mL of deionized water and stirred magnetically to obtain a homogeneous mixture. The three

solutions were combined in a 100 mL volumetric flask, diluted with deionized water and mixed. A pure glucose standard stock solution of 2,025 ppm was prepared by dissolving 0.405 g of pure glucose in 100.0 mL deionized water. The solution was transferred to a 200.0 mL volumetric flask and diluted. Standards were prepared from the stock solution. For example, a 69.9 ppm standard was prepared by adding 0.209 g of the glucose stock solution to 1.02 g DNS reagent and 4.82 g of deionized water. Other standards were prepared as listed in Table 2.

Table 2. Standards for calibration of the DNS method

Sample	DNS Reagent (g)	Glucose Standard (g)	Water (g)	Conc. Of Glucose (ppm)
1	1.020	0.209	4.820	69.90
2	1.022	0.406	4.691	135.0
3	1.014	0.608	4.425	203.0
4	1.030	0.819	4.252	272.0
5	1.018	1.004	4.023	336.6
6	1.014	1.222	3.832	406.7
7	1.030	1.439	3.635	477.8
8	1.025	1.607	3.421	539.8
9	1.001	1.829	3.211	612.1
10	1.011	2.019	3.022	676.0
Blank	1.013	0	5.007	0

Separate sets of standard solutions were heated for 5, 20 and 40 minutes at a constant temperature of 100 °C to determine the effect of heating time on the calibration. After heating, the samples were cooled under cold running water for about 20 mins. The absorbance of the samples were measured at 540.6 nm and a calibration curve was generated.

Determination of Total Organic Carbon in the Hydrolyzed Samples

Approximately 10 g of each hydrolyzed filtrate was transferred to an Erlenmeyer flask followed by the addition of 0.4 mL buffer solution (pH = 2). The samples were stirred for 10 minutes. About 3.00 ml of each sample was transferred into three separate digestion vials

containing 0.1 g of persulfate as an oxidizing agent. The blue indicator ampules were placed in each sample and then the digestion vials were capped tightly. The samples were heated in the DRB 200 reactor for 2 hours at a constant temperature of 105 °C followed by cooling for 1 hour. A UV-Vis Spectrovis Plus Spectrophotometer and Logger Pro 3 software was used to measure the absorbance of the blue indicator solution (λ_{max} 591.8 nm). The total organic carbon present in Arundo donax hydrolyzed at 100 °C and cellulose samples hydrolyzed (40 °C, 60 °C, 80 °C, and 100 °C) were measured by this method.

DNS Assay of Hydrolyzed Cellulose and Arundo Donax at 100 °C

The DNS method was used to determine the amount of reducible sugars present following hydrolysis of microcrystalline cellulose and Arundo donax. For DNS analysis, a total volume of 6 mL of each sample was prepared using micropipettes. Dinitrosalycylic acid assay reagent (1,000 µL) and deionized water (3,500 µL) was added to three filtered samples (1,500 µL) obtained carrying out hydrolysis of microcrystalline cellulose and Arundo donax. The samples were heated for 40 minutes at 100 °C and subsequently cooled under cold running water for 20 minutes. The absorbance of each sample was measured at a wavelength of 540.6 nm and the amount of glucose was determined. This procedure was used to quantify the amount of reducible sugars obtained from enzymatic hydrolysis of cellulose and Arundo donax.

CHAPTER 3

RESULTS

Total Organic Carbon (TOC) Method Calibration of Hach DRB 200-3 Reactor

To calibrate the TOC method, potassium hydrogen phthalate (KHP) was used as a primary standard. After the TOC method on each standard and hydrolyzed sample, there was a decrease in the color intensity of the blue indicator used in the TOC method while the color intensity of the blank sample remained virtually the same. Figure 8 presents the color change of the blue indicator before and after TOC method carried out on the hydrolyzed Potassium Hydrogen Phthalate (KHP) standard.

Figure 8. Color of indicator before and after TOC method

Figure 9 presents the visible spectrum of the indicator solution before being used in the TOC method. The peak was found to have maximum absorbance at a wavelength of 591.8 nm. The difference between the absorbance before and after the method represents the change in absorbance which is proportional to total organic carbon in units of ppm KHP. The calibration is shown in Figure 10 with an acceptable linear correlation of 0.9951.

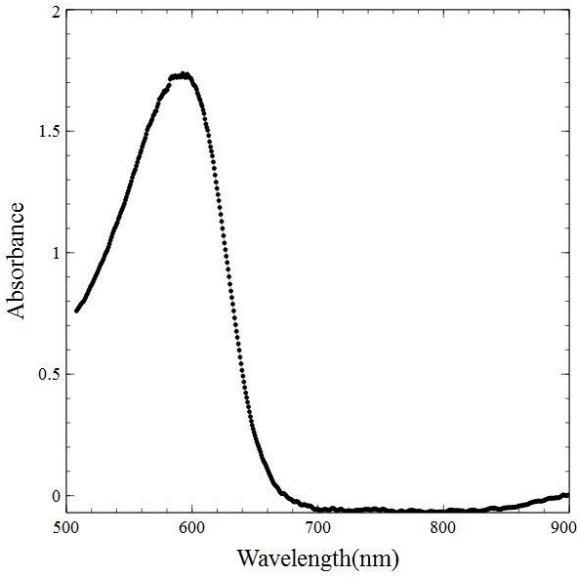

Figure 9. Visible spectrum of total organic carbon indicator

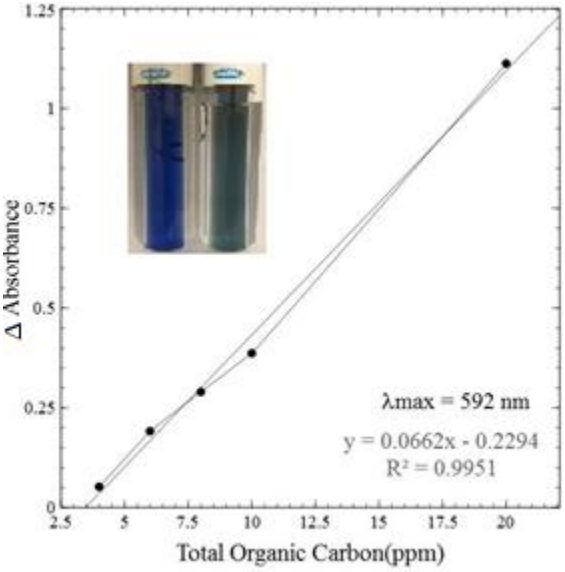

Figure 10. Calibration curve for total organic carbon using KHP standard solutions.

Total Organic Carbon in Microcrystalline Cellulose

The amount of TOC obtained from the hydrolyzed microcrystalline cellulose using concentrated sulfuric acid and blue molybdenum at various temperatures are given in Table 3. Based on the dilutions described, the amount of TOC is reported in milligrams (mg). As

expected, the highest amount of total organic carbon was obtained when hydrolysis is carried out at 100 ^{o}C.

Table 3. The amount of TOC from sulfuric acid and BM hydrolysis

Temperature (^{o}C)	Sulfuric Acid (mg)	Error (±mg)	BM (mg)	Error (±mg)
40	0.801	0.008	0.66	0.01
60	0.932	0.037	0.80	0.02
80	1.287	0.015	1.04	0.05
100	1.62	0.23	2.0	0.2

Hydrolyzed microcrystalline samples catalyzed by H_2SO_4 and BM at 100 ^{o}C produced statistically similar amounts of total organic carbon.

Thin-Layer Chromatography (TLC) Results

Thin-layer chromatography was used in this work in an attempt to separate and quantify glucose and other sugars produced as a result of acid hydrolysis. A typical TLC plate is shown in Figure 11.

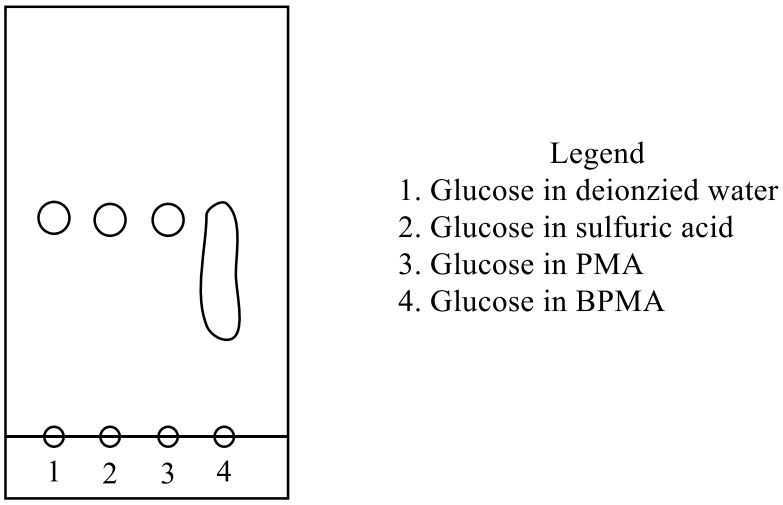

Figure 11. TLC spot map of neutralized samples

The TLC spot maps of the neutralized glucose standards produced clear spots while sample 4 produced a long blue streak which made it difficult to identify a distinct spot of glucose. The long streak is most likely due to a mix of molybdenum species with different oxidation states. Hence, TLC was not an efficient method to separate and quantify glucose obtained from the hydrolyzed cellulose. This led to using the dintirosalicylic assay method (DNS).

Calibration of Dinitrosalicylic Method

In this method a positive result for a reducing sugar is obtained when there is a color change from yellow (3,5-dinitrosalicylic acid) to red-brown which is 3-amino-5-nitrosalicylic acid.[34] This color change is shown in Figure 12.

Figure 12. Color change before and after DNS method

The DNS method was carried out using glucose standard solutions ranging from 100-700 ppm. Combined with DNS the samples was heated at 100 ^{o}C for 5 mins, 20 mins, and 40 minutes resulting in the calibration curves for reducible sugars shown in Figures 13, 14 and 15.

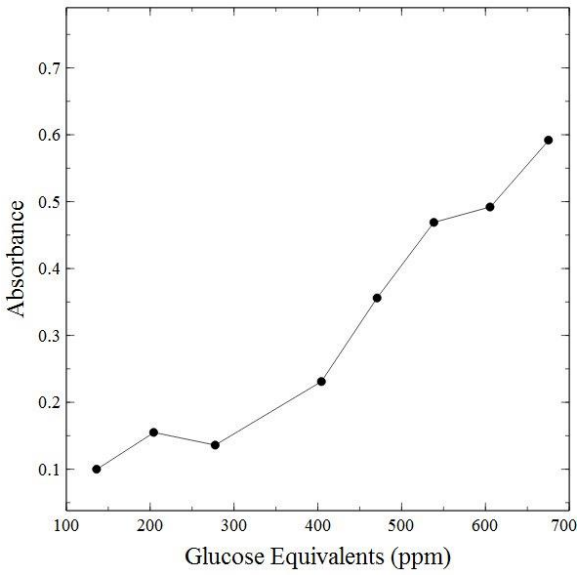

Figure 13. Calibration for reducible sugars using pure glucose and heating for 5 minutes

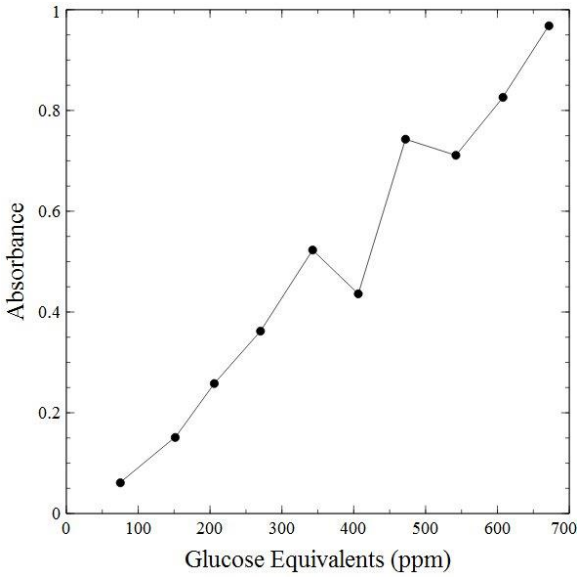

Figure 14. Calibration for reducible sugars using pure glucose and heating for 20 minutes

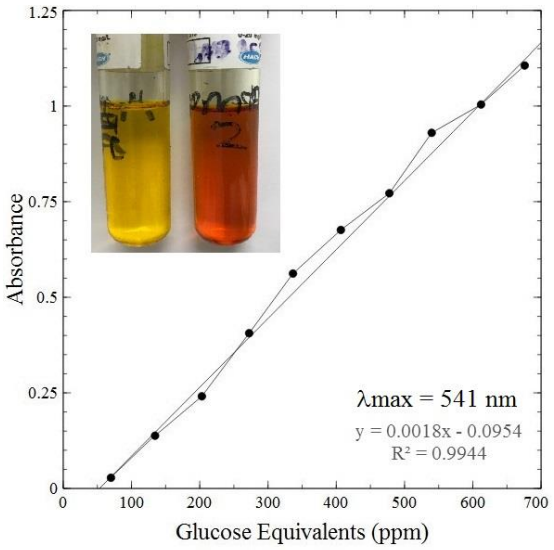

Figure 15. Calibration for reducible sugars using pure glucose and heating for 40 minutes

Heating for 40 minutes produced a calibration curve with the most acceptable linear regression for the quantification of glucose in the samples analyzed.

Glucose from Acid Hydrolysis

The amount of glucose in milligrams (mg) obtained hydrolyzing microcrystalline cellulose at 100 ^{o}C using water, PMA, and BM catalysts are provided in Table 4.

Table 4. Amount of reducible sugars after hydrolysis of microcrystalline cellulose at 100 ^{o}C

Catalyst	Glucose (mg)	Error (±mg)
H_2O (Blank)	10.9	0.2
BM (Blank)	17	2
H_2SO_4	9.9	0.7
PMA	10.7	0.6
BM	20	2

Unfortunately, use of sulfuric acid, PMA or BMA did not result in a statistically higher amount of glucose indicating acid hydrolysis of microcrystalline cellulose was not successful. The same is true when using powdered Arundo donax and these results are not presented. Even using concentrations of sulfuric acid up to 6.0 M did not result in a positive result for glucose using the DNS method. One possible reason for this could be due to the microscale nature of the experiments.

Glucose from Enzymatic Hydrolysis

Table 5 shows the amount of glucose obtained using cellulase to carry out enzymatic hydrolysis of microcrystalline cellulose and Arundo donax.

Table 5. Enzymatic hydrolysis of cellulose and Arundo donax

Sample	Glucose (mg)	Error (±mg)	Sample	Glucose (mg)	Error (±mg)
Cellulose	31	2	Arundo donax	48	9
Cellulose and cellulase	162	3	Arundo donax and cellulase	163	2

The milligram amount of glucose obtained using microcrystalline cellulose and Arundo donax corresponds to a weight percent yield of 26.2 and 23.0% percent respectively.

CHAPTER 4

DISCUSSION

The amount of TOC in the various samples analyzed was a means to assess the efficiency of degradation of the microcrystalline cellulose catalyzed by sulfuric acid, and BM at low concentrations and similar pH conditions. The BM reagent used has a pH of 3.31 compared to sulfuric acid measured to be 3.27. The TOC method produces carbon dioxide as a result of the oxidation of the samples by persulfate. The carbon dioxide reacts with the blue thymol indicator to form carbonic acid which leads to the reduction in the color intensity from deep blue to pale blue. The change in color intensity of the indicator results in the change in absorbance which is directly proportional to the quantity of soluble organic carbon in the sample analyzed. After the TOC method on the blank sample which contained deionized water only, the color of the blue indicator remained virtually identical. After running the TOC method on a sample of hydrolyzed cellulose, the change in absorbance accounts for the amount of total organic carbon present. The amount of total organic carbon increased with temperature as expected. This was due to the increased rate of hydrolysis. The weakening of the crystallinity of the microcrystalline cellulose enhanced protonation of the hydrogen bond network resulting in reducible and non-reducible sugars. At a temperature of 100 ^{o}C, a statistically similar amount of TOC was obtained for sulfuric acid and BM.

An inexpensive separation method to quantify the reducible sugars is always desirable. The TLC method used by Nam Soo et al. was modified to achieve this goal. Nam Soo et al. applied TLC to separate aldoses and their corresponding alditols such as D-glucose and D-glucitol within a detection range of 500 ng to 1 µg.[33] To assess the feasibility of separation of un-neutralized reducible sugars by chromatography, TLC was performed on pure glucose

dissolved in deionized water, sulfuric acid, phosphomolybdic acid and blue molybdenum as a control experiment. No TLC spots were observed after eluting the samples by a solvent system constituting 1-butanol, ethyl acetate and water in the volume ratio of 8:1:1. This could be attributed to the fact that the reducible sugars in solution were protonated leading to the formation of charged species which interacted strongly with the mobile phase. The charged molecules travelled with the solvent front making separation impossible. TLC carried out glucose samples dissolved in both acid catalysts and after neutralization resulted in a positive spot for glucose. This confirmed the ability to separate neutralized samples by TLC. No distinct spot was obtained for the pure glucose standard dissolved in the blue molybdenum solution but rather a long blue streak emanating from the color of the blue molybdenum. TLC performed on the neutralized hydrolyzed microcrystalline cellulose samples did not produce any visible spot most likely due to the concentration of reducible sugars being lower than the detection limit of this method. Unfortunately, TLC was not a simple or efficient method to separate the reducible sugars obtained in this work.

The use of the DNS method as an alternative proved to be fast and more precise compared to the TLC method to determine the amount of reducing sugars. The DNS method was used because it was less time consuming compared to GC and HPLC. Although, trials are ongoing using HPLC to identify different sugars present. The duration of heating in DNS methods reported previously involved heating the samples in a thermocycler for about 2 to 10 minutes. This work found that 40 minutes was required to obtain a full coloration of the red-brown 3-amino-5-nitrosalicylic acid. Heating for 40 minutes resulted in a plot of absorbance and concentration with the best linear fit. The increased amount of time required to heat the sample compared to what has been reported in literature (2 to 10 mins) could be attributed to heat loss

from the DRB reactor due to having a plastic cover. The amount of reducible sugars obtained from the hydrolyzed cellulose at 100 ^{o}C was statistically similar when using water, sulfuric acid and PMA. Surprisingly, a larger amount of glucose was obtained when BM was used compared to water, sulfuric acid and PMA. However, when compared to a BM blank, the amount glucose obtained was the same indicating that BM does not carry out acid hydrolysis of cellulose. One possible cause of these results is the concentration of the acid. Even using sulfuric acid up to 6.0 M did not result in measurable glucose using the DNS method.

Enzymatic hydrolysis was most successful for converting cellulose to glucose. Nearly identical amounts of glucose were obtained using Arundo donax and microcrystalline cellulose. This is a very interesting result because the Arundo donax did not require any pretreatment such as a steam explosion or chemical modification. Further research of this system is planned including understanding the effect of blending time and experiments to determine enzyme kinetics of this system.

Conclusion

The catalytic activity of blue molybdenum towards the degradation of microcrystalline cellulose to produce reducible and non-reducible sugars as an inexpensive source of biofuel precursors has been investigated. The quantification of total organic carbon and reducible sugars in the hydrolyzed Arundo donax catalyzed by BM using the TOC and DNS showed that acid hydrolysis producing glucose was not successful. Statistically similar amounts of reducible sugars was obtained hydrolyzing microcrystalline cellulose at 100 ^{o}C using water, PMA, BM and sulfuric acid. However, enzymatic hydrolysis of Arundo donax was successful resulting in a 26% yield for glucose.

Future Work

Future work includes determining the amount of glucose generated when carrying out enzymatic hydrolysis of Arundo donax over time. Additionally, the effect of pretreatment methods are planned. Examples include steam explosion and oxidation. Forms of oxidation include the use of Fenton's reagent which generates highly oxidizing hydroxyl radicals. Instead of chemical oxidation, microbial degradation is another form of pretreatment. Trials examining microbiological production of glucose from carboxymethyl cellulose and in combination with hydrolysis using cellulase are ongoing.

REFERENCES

1. Jessica, H.; Kyle, C.P.; Brenton, B.; Brett, L.L. Conversion of cellulose to glucose and levunic acid via solid-supported acid catalyst. Elsevier, Tetrahedron Letters, 51, 2356-2358, **2010**
2. Huber, G.W.; Ibora, S.; Corma, A. Chem Rev. 106, 4044, **2006**
3. Ayhan, D. Political, economic and environmental impacts of biodiesels: A Review. Elsevier, Applied Energy, 86, S108-S117, **2009**
4. Akash, N. Dissolution of cellulose. ProQuest LLC. 2. **2010**
5. Feng J, Qingyun Z, Ding M, Xiumei L, Xiuwen H.; Direct conversion and NMR observation of cellulose to glucose and 5-hydroxymethylfurfural catalyzed by acidic anionic liquid. J. molecular catalysis, 334, 8-12. **2011**
6. Klemm D, Heublein B, Fink H.P., Bohn A.; Cellulose, Fascinating biopolymer and sustainable raw material. Angew chem. Int. Ed. 44 (22), 3358-93. **2005**
7. Fan L.T., Gharpuray M.M., and Lee Y.H.; Cellulose hydrolysis. Springer-verlag, Berling. **1987**
8. Caijian Li; Enzymatic hydrolysis of cellulose from various waste sources and their feasibility as feedstocks for ethanol production. Heritage Branch. 22. **2004**
9. Wang Y, Zhao Y, Deng Y.; Effect of enzymatic treatment on cotton fiber dissolution in NaOH/Urea solution at cold temperature. carbohydrate polymer, 72, 178-184, **2008**
10. Davison B.H, Ragauskas A.J, Templer R, Tschapplinski T.K, Melenz J.R.; Measuring the efficiency of biomass energy. Science. 312, 1744, **2006**
11. Fukuoka A, Dhepe P.L.; Catalytic conversion of cellulose into sugar alcohols. Angew chem. Int. Ed. 45, 5161. **2006**

12. Yadav J.S, Satyanarayana M, Balanarsaiah E, Raghavendra S.; Phosphomolybdic acid supported on silica gel: a mild, efficient and reusable catalyst for the synthesis of 2,3-unsaturated glycopyranosides by Ferrier rearrangement, Tetrahedron letters, 47, 6095-6098, **2006**
13. Luca C., Roberto P., and Fabrizio A.; Arundo donax: A non-food crop for bioenergy and bio-compound production. Biotechnology advances 32, 1535-1549, **2014**
14. Mariani C., Cabrini R., et al; Origin, diffusion and reproduction of the giant reed (Arundo donax L): a promising weedy energy crop. Ann Appl. Biol, 157. 191-202. **2010**
15. Papazoglou E.G., Karantounias G.A., Vemmos S.N., Bouranis D.L.; Photosynthesis and growth responses of giant reed to the heavy metals Cd and Ni. Environ Int. 31, 243-249. **2005**
16. Akash Narani; Dissolution of cellulose. ProQuest LLC.2. **2010**
17. Jessica Heigner; Conversion of cellulose into biofuel precursors via solid supported acid catalysis. ProQuest LLC. 1,2. **2012**
18. El-Wakil N.A, Hassan M.L.; Structural changes of regenerated cellulose dissolved in FeTNa, NaOH/thiourea and NMMO systems. J. Appl. Poly. Sci. 109 (5), 2871. **2008**
19. Cai J, Zhang L.; Rapid dissolution of cellulose in LiOH/urea and NaOH/urea aqueous solutions. Macromol. Biosci. 5 (6), 539-548. **2005**
20. Luo C, Wang S, Liu H.; Cellulose conversion into polyols catalyzed reversibly formed acids and supported ruthenium clusters in hot water. Angew. Chem. Int. Ed. 46, 7636. **2007**
21. Badger P.C.; "Ethanol from cellulose: A General Review". Trends in new crops and new uses. ASHS Press. **2002**

22. Saeman J.F.; Kinetics of wood saccharification. Industrial and engineering chemistry. 37, 43. **1945**

23. Bert A. E.; Three stage hydrolysis of cellulose. ProQuest. LLC. 8. **1974**

24. Krassig H., Schurz J., Steadman R.G., Schliefer k., Albrecht W., Mohring M and Scholsser H; Cellulose, Ullman;s Encyclopedia of Industrial Chemistry, Wiley-VHC, Weinheim, **2007**

25. Lanzafame P, Temi D.M. Parathoner S, Spadora A.N., Centi G.; Direct conversion of cellulose to glucose and valuable intermediates in mild reaction conditions over solid acid catalysis. Catalysis Today. 179, 178-189. **2012**

26. Jizhe Z., Xin L., Miao S., Xiaohua M and Yu H.; Direct conversion of cellulose to glycolic acid with a phoshomolybdic acid catalyst in a water medium. ACS catalysis, 2, 1698-1702, **2012**

27. Geboers J., Van V.S., Carpentier K., Jacobs P. and Sels B.; Green Chem. 13, 2167. **2011**

28. Sun N., Jiang X.Y., Maxim M.L., Metlen A., and Rogers R.D.; ChemSusChem. 4, 65. **2011**

29. Edward A.N., Ian D.M., Paul W., Spas D.K.; The blue molybdenum reaction for the determination of orthophosphate revisited: Opening the black box. J. ACA, 890, 60-80, **2015**

30. Stephan Enthaler and Xiao-Feng Wu; Zinc catalysis: Application in organic synthesis, First Edition, Wiley-VCH Verlag GmbH, Weinheim, 1-3, **2015**

31. Total organic carbon direct method 10129, DR 800 LR, http://www.hach.com/total-organic-carbon-toc-reagent-set-lr/product-downloads?id=7640196847, 03:28 PM, 02/01/2017

32. Nam S.H. and John F.R.; Separation and detection of sugars and alditols on thin-layer chromatograms, carbohydrate research, 313, 135-137, **1998**

33. Ian P.W., Adam E., Peter R., Ian B., Ian N. R., Keith W.W.; Rapid quantification of reducing sugars in biomass hydrolysates: Improving the speed and precision of the dinitrosalicylic acid assay. Biomass and Bioenergy, **44, 117-121**, **2012**

VITA

FAROUK AWUDU

Education:	MS Chemistry, East Tennessee State University, Tennessee, USA, (Aug 2018)
	B.Sc. Chemistry, Kwame Nkrumah University of Science and Technology, Ghana, (Aug 2004-May 2008)
Work Experience:	Graduate Teaching Assistant, Department of Chemistry, East Tennessee State University, (August 2016-May 2018)
	Regulatory Officer, Ghana Food and Drugs Authority, Tema, Ghana (November 2010-December 2015)
	Teaching Assistant, University of Ghana, Department of Chemistry, Ghana (July 2008 - Aug 2009)
Research Experience:	Graduate research student, East Tennessee State University, Tennessee, USA, Research Supervisor: Dr. Dane W. Scott (Jan 2016-Aug 2018)
	Undergraduate research student, Kwame Nkrumah University of Science and Technology, Research Supervisor: Dr. Ray Voegbolor (Aug 2004-May 2008)
Presentations:	Farouk Awudu, Dr. Dane W. Scott, Clair Baker, Troy Dolmetsch, Silmon Joshua

Hydrolysis of cellulose and biomass using phosphomolybdic acid and blue molybdenum, Graduate Seminar, East Tennessee State University (March 2018)

Farouk Awudu, Dr. Ray Voegborlo. Isolation and purification of some known organic compounds in the fruits of Piper guineense, Kwame Nkrumah University of Science and Technology (April 2008)

Honors and Awards: Outstanding Teaching Assistant Award, Department of Chemistry, East Tennessee State University (Nov 2017)