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Phosphomolybdic Acid Catalysis of Cellulose Hydrolysis

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Abstract

Renewable sources such as cellulose derived biofuels are sought after in order to replace fossil fuel sources that are currently used to meet energy demands. Cellulose is a biological polymer composed of a chain of glucose molecules. Hydrolysis of cellulosic materials then has potential to serve as a source of renewable energy in the form of biofuels. The crystalline structure of cellulose is very stable, and current methods of catalyzed hydrolysis are inefficient for industrial application. This project explores the use of phosphomolybdic acid (PMA) in water to catalyze hydrolysis of microcrystalline cellulose. Temperature of hydrolysis was varied from 40 °C – 100 °C. The amount of soluble hydrolysis product was determined through wet oxidative total organic carbon analysis using a Hach method kit. Total organic carbon content is compared between equimolar amounts of PMA and sulfuric acid, the current industry preference. The yield of total organic carbon in parts per thousand (ppt) is directly correlated to increasing temperatures. Across these temperatures, PMA is more efficient than sulfuric acid in hydrolysis of cellulosic materials. Work is ongoing for glucose-specific product detection as well as evaluating the recyclability of the catalyst.

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1. Introduction

Humans continued reliance on fossil fuels to meet energy demands in a post-industrial age has led to the alarming depletion rate of fossil fuel reserves. Estimates according to Ecotricity, an environmentally conscious energy company based in Britain, provides that current reserved levels of oil will be depleted early in the 2050's decade. [1] It is also anticipated that this depletion of crude oil would induce further reliance on other fossil fuels such as coal. Although coal has the most plentiful reserves of the fossil fuels, if we continue to rely on fossil fuels for energy it could also be depleted before the end of the century due to a growing global urban population and amplified dependence. [1] Not only are fossil fuels problematic due to their inevitable exhaustion, but they also have well-known negative environmental impacts as well. The direct burning of fossil fuels release CO_2, which is a major cause of climate change. [2] Therefore, investigating the applications of alternative fuels that are both sustainable and environmentally-friendly is of the utmost importance in research.

In the class of alternative fuels, biomass derived biofuels are especially promising, but require further development. The traditional use of starch biofuels such as those derived from corn biomass has enjoyed widespread application for its ease of chemical conversion, but suffers from a range of drawbacks as well. Cellulosic biofuels offer competitive advantages over those derived from starch, but the difficulty of converting cellulose into fuel products has slowed its widespread application. Cellulose attracts the attention it does because it is abundant, inexpensive, and is a polymer of hundreds to even thousands of individual glucose monomers. Therefore, the conversion of hydrolyzed cellulose sugars into fuel compounds holds the potential to cheaply yield a substantial amount of chemical energy. Conversely, the hydrolysis of cellulose has proven to be difficult due to its crystalline structure, which demonstrates stability across a

wide range of conditions. Current technology uses either enzymatic or chemical methods to catalyze the hydrolysis. Of these methods, investigation into developing new chemical hydrolysis methods offers the most opportunity for advancing the field. In an attempt to improve upon the chemical methods of catalysis, this project examines the use of catalyzing microcrystalline cellulose with phosphomolybdic acid (PMA). Systematic temperature trials of hydrolysis will evaluate catalyst efficiency and offer information about procedure optimization in terms of minimizing energy input.

2. Background

2.1 Biofuels

Biofuels are non-fossil fuels produced from captured solar energy that is stored as chemical energy in the form of organic compounds by organisms. [2] This capture and storage process involves carbon fixation of CO_2 into sugars through the process of photosynthesis. Microorganisms are utilized to transform the sugars into useful fuel compounds including ethanol and other short- to medium-chain alcohols, as well as organic acids and alkanes/alkenes.[3] Figure 1, below, provides a generic schematic to the production of fuels from biomass: [4]
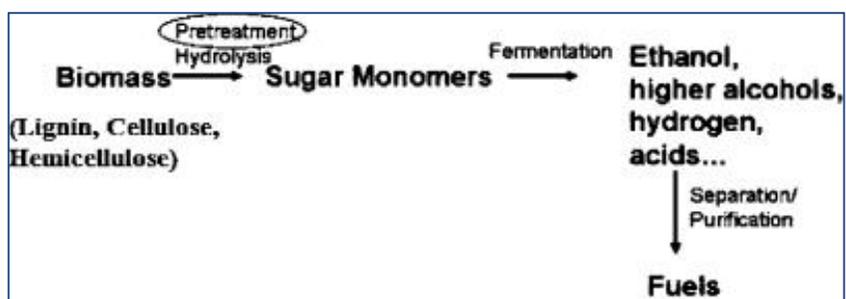
Figure 1: Schematic of the example conversion of cellulosic biomass to fuel. [4]

The burning of these biofuels re-releases CO_2 back into the atmosphere, which as a closed CO_2 cycle is more carbon-neutral than the direct burning of fossil fuels.[2] Compared to current fossil fuel energy sources, cellulosic ethanol and ethanol produced from other biomass resources have the potential to cut greenhouse gas emissions by 86%.[5]

Common feedstocks in the production of biofuels rely on either starch or cellulosic materials. Of the two, biofuels from starch such as corn kernels is the more developed and exploited of the two. The use of corn as a feedstock, however, presents major drawbacks. [2] As a crop, corn requires a massive allocation of resources such as large land usage and vast quantities of water. Additionally, the heavy usage of fertilizers and pesticides pose environmental hazards. Lastly, the use of corn as a fuel feedstock competes with its role in food supply. The uncertainty of whether feeding a growing global population or meeting energy demands should take priority is referred to as the "food vs. fuel" debate, and has complicated the distribution of resources. [6]

On the other hand, cellulose derived biofuels offer the advantages of cellulosic materials being abundant, inexpensive and largely inedible for humans.[7] Although currently less commonly used as a feedstock in biofuels, cellulose contains the same potential chemical energy as starch materials. Given the relative abundance of cellulosic materials, the wealth of chemical energy that cellulose offers is best described by Hu et al.: [7]

> "[…] Nature produces 200 billion metric tons of lignocellulosic
> biomass with an energy content of 3×1018 kJ year^{-1} by
> photosynthesis, which is around 10 times the present and annual energy
> consumption of the world"

The majority of this potential chemical energy, however, has yet to been realized. Although cellulose offers considerable advantages over starch, starch has traditionally been used for the

production of biofuels for the relative ease by which starch is hydrolyzed to sugars and converted into fuel compounds. Cellulose, however, is an extremely stable polymer that is comparatively much more difficult to breakdown than starch.

2.2 Cellulose

Cellulose is found naturally in the cell wall of plant cells, and is well known to give plant cells their structural rigidity. Plants store their carbohydrates in many lignocellulose derivatives such as cellulose, hemicellulose and lignin, but this review will focus only on that of the cellulose structure.[8] Cellulose is comprised of hundreds to thousands of D-glucose monomers that are linked together through a β-(1→4)-glycosidic bond. An example structure of cellulose is provided in Figure 2 (below):
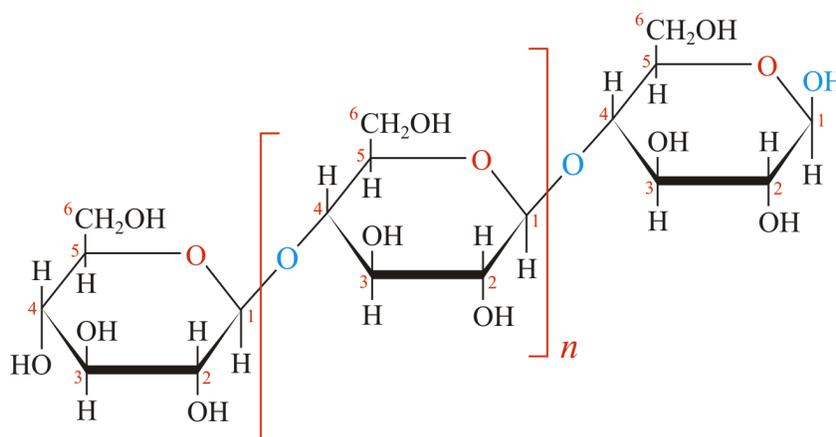
Figure 2: Three glucose molecules linked through β-(1→4)-glycosidic bond bonds to give an example of the basic repeating structure of cellulose.

Unlike other sugar polymers, cellulose is a straight-chain polymer that is largely unbranched.[9] Therefore, in its simplest form, cellulose exists structurally as a crystalline compound. The glucose molecules of cellulose, however, contain hydroxyl groups with the capacity to hydrogen bond. Hydrogen bonds may be formed between different chains of cellulose, as well as intrachain interactions.[10] Stabilization of the polymer through hydrogen bond interactions

induces formation of many of the chains into microfibrils that aggregate to form larger fibrils.[11]

The tendency to form the fibril state is key to the rigid character and high tensile strength of

cellulose. These properties provide the protective and supportive functioning of cellulose within

the walls of plant cells. Unfortunately, the hydrolysis of cellulose into individual glucose

monomers is complicated by the stable nature of the polymer. Ultimately, catalysts and/or harsh

reaction conditions are required in order to induce hydrolysis to yield glucose monomers.

2.3 Cellulose Hydrolysis by Enzymatic Methods

One major mechanism of cellulose hydrolysis makes use of biological enzymes.

These cellulose-hydrolyzing enzymes, referred to as cellulases, are derived from microorganisms

such as bacteria or fungi. Much is still unknown about the mechanisms by which these cellulases

accomplish hydrolysis, but it is understood that bacterial cellulases form an enzyme system that

work synergistically. The system requires the action of at least three enzymatic components

consisting of endoglucanases, exoglucanases and β-glucosidases and possibly others.[12] Given the

biological nature of these enzymes, cellulases are sensitive to conditions such as temperature,

pH, and concentration of inhibitors.[12] Therefore, physiological parameters are important

consideration in the enzymatic hydrolysis of cellulose.

In its application to industry, enzymatic hydrolysis is the preferred industrial method of

cellulose hydrolysis over chemical systems.[13] Cellulases offer the advantage of being selective.

This selectivity minimizes undesired hydrolysis products and simplifies fermentation processes.

Another advantage of enzymatic hydrolysis are the mild reaction conditions. Despite these

advantages, by no means is enzymatic hydrolysis a perfect process. One major drawback is that

the rate of enzymatic cellulose hydrolysis is slow. It is well reported that cellulase-cellulose

interactions demonstrate a rapid hydrolysis rates at first followed by a significant decrease in

conversion rates over time for reasons not completely understood.[14] As mentioned, cellulases are

sensitive to physiological conditions and attempts to further catalyze their functioning through

temperature or pH changes would be counter-productive. The simple solution is to increase the

concentration of enzymes relative to cellulose. For industrial hydrolysis, the typical ratio of

cellulase to cellulose is 25 g:1 kg.[15] Given the high concentration of cellulases required,

enzymatic hydrolysis becomes a numbers game in which simply not enough cellulases are

produced to support industrial needs. Strain improvement by mutagenesis has been researched to

improve cellulase activity of certain organisms, but efforts to develop microorganism strains that

produce higher amounts of cellulase has been largely unsuccessful. [12] Essentially, these

considerations point to the fact that enzymatic cellulose hydrolysis is expensive. Biofuels derived

from cellulose are more costly than those made from starch, and hydrolysis of cellulose becomes

the bottleneck of bringing down the cost of cellulosic biofuels. In the four-step production of

biofuels from cellulose, the enzymatic hydrolysis requires half of the total cost, in which

cellulases are the most expensive part, comprising up to 25% of total costs.[16]

2.4 Cellulose Hydrolysis by Chemical Methods

The traditional approach to cellulose hydrolysis by chemical methods is characterized by

the use of strong acids under high temperatures and pressures. In simple hydrolysis of water,

high temperature (>300 C) and pressure (25 MPa) are needed to compromise the crystalline

structure to the amorphous form.[17] The implementation of a concentrated chemical catalyst under

similar high temperature and pressure conditions results in sufficient hydrolysis yields. Of the

chemical catalysts, mineral acids such as hydrochloric acid (HCl), nitric acid (HNO_3) and

sulfuric acid (H_2SO_4) are commonly used.[7] These chemical catalysts are then recycled, as such

for the patented Arkenol technology which uses and recovers and reconcentrates H_2SO_4 for the

hydrolysis steps.[18] Current technology in the field of acid catalyzed hydrolysis, however, is shifting from homogenous acids to heterogeneous solid acid catalyst for the ease in which they are separated from the product solution.[19] Another important consideration in the chemical hydrolysis of cellulose is the accessibility of the cellulose in solution. As it is an unbranched polymer, cellulose demonstrates poor solubility in aqueous solutions. Therefore, some chemical methods of catalysis utilize expensive ionic liquids as the solvent system, in which cellulose is soluble.[20]

Overall, chemical methods of cellulose hydrolysis offer both advantages and disadvantages. One of the advantages is that chemical methods demonstrate good catalytic activities that improve upon hydrolysis rates from enzymatic hydrolysis reactions.[7] Also, chemical methods are inexpensive.[7] On the other hand, chemical methods have the disadvantage of traditionally involving harsh conditions that are energy-expensive. Additionally, the use of concentrated acids leads to reactor corrosion as well as waste products that are difficult to treat.[21] Lastly, the use of concentrated acids is often less selective and can produce undesired byproducts that complicate future fermentation steps and negatively impact potential yields.[21] Humins, or insoluble organic compounds formed from the condensation of sugar intermediates, are especially difficult to avoid in conventional chemical hydrolysis methods. [22] Therefore, the investigation into a chemical catalyst that requires milder conditions, demonstrates higher selectivity, and offers enhanced cellulose solubility properties would advance current chemical hydrolysis technology.

2.5 Phosphomolybdic acid

Phosphomolybdic acid (PMA) has the chemical formula of $H_3PMo_{12}O_{40}$. Figure 3, below demonstrates the chemical structure of this coordinated compound: [23]
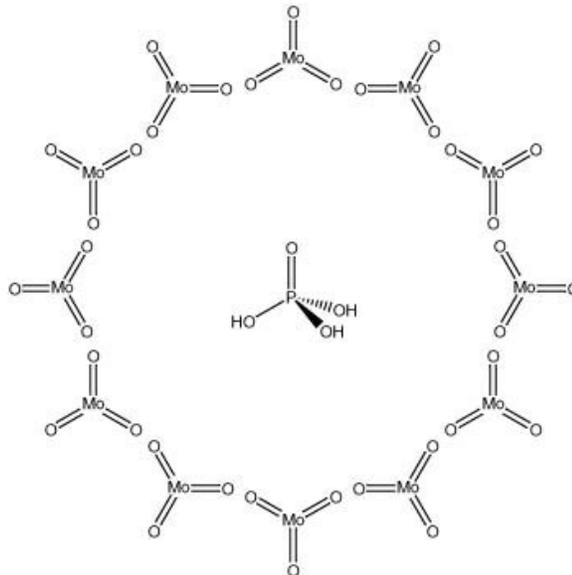
Figure 3: The chemical structure of H3PMo12O40 phosphomolybdic acid (PMA).[23]

In this form, PMA is a yellow compound, although other versions of this coordinated compound demonstrate alternative physical properties. PMA is a heteropoly acid, which offers desirable solubility properties both in water and for the dissolution of cellulose.[20] Results in other labs show early indication that molybdenum atoms in this coordinated compound favor selective oxidation of cellulose.[20]

2.6 Total Organic Carbon (TOC) analysis

Glucose-specific detection tests are both complicated and labor-intensive.[24] Instead, the procedure we used utilizes a total organic carbon (TOC) analysis approach to approximate the amount of products present after hydrolysis with PMA catalyst. While there are several possible procedures to accomplish TOC measurements, the general approach provides information about the amount of total organic carbon that is present in the system. Our experiment will follow the common TOC procedure of converting organic carbon into carbon dioxide for the subsequent quantification.[25] The amount of TOC is especially useful in instances of wastewater treatment

and water purification. These industries make use of TOC in order to preliminarily estimate the amount of organic contaminants present in their complicated samples while using simple procedures and favorable cost considerations.[26] Due to the fact that the content input in our experimental hydrolysis systems may be controlled, total organic carbon content should therefore directly correlate to the concentration of hydrolyzed sugar products.

3. Methodology

3.1 Research Design

This project is concerned with evaluating the ability of phosphomolybdic acid to catalyze hydrolysis of cellulose. As such, the design of the research experiment involves utilizing phosphomolybdic acid dissolved in water to carry out a hydrolysis reaction at varying given temperatures. For instance, the first procedures involve the collection of data for a catalyzed hydrolysis at 40 °C, 60 °C, 80 °C and 100 °C trials. Scheme 1, below, details the proposed reaction:
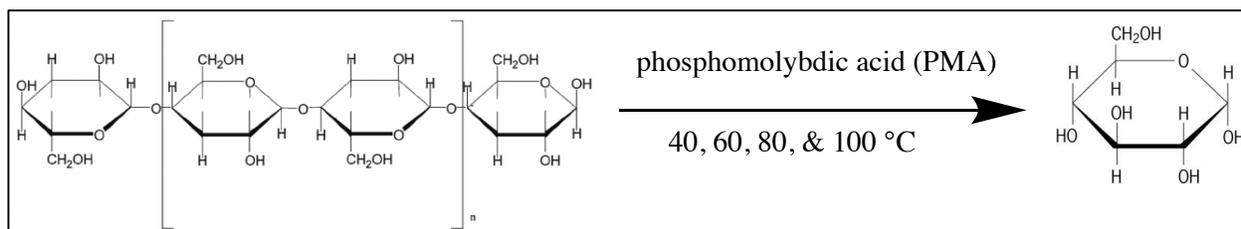
Scheme 1: Proposed hydrolysis of cellulose to glucose using a PMA catalyst.

After this, catalyzed hydrolyses are analyzed using a total organic carbon (TOC) analysis method.

The TOC procedure is founded on the principle that organic carbon is oxidized to carbon dioxide when digested with persulfate. In the TOC procedure, hydrolyzed cellulose products are placed in specialized vials that are compatible for reaction inside the digester instrument. During digestion, organic carbon reacts with persulfate to form carbon dioxide which is released from

the solution in the form of vapor. The capped vial, however, forces dissolution of the carbon

dioxide into an indicator solution in an ampule. Inside the ampule, carbon dioxide reacts to form

carbonic acid, which induces a color change. An example of the experimental vial/ampule

system is included below in Figure 4. The resulting indicator solution in the ampule is then

analyzed by uv-vis spectrophotometry to quantify the degree of the color change of the

indicating solution as a result of carbonic acid dissolution. The resulting absorbance of the

solution is compared against an experimentally developed standardization curve in order to

determine the concentration of organic carbon after hydrolysis relative to the amount of catalyst

used.

Figure 4: Picture of the experimental vial/ampule system.

3.2 Variables and Measurements

The indicating solution found in the ampule naturally yields high readings of absorbance

measurements. After TOC procedure, lower absorbance measurements indicate the presence of

organic carbon. This is because under pressure gaseous carbon dioxide dissolves in the indicating

solution that produces a colorimetric chemical reaction. The absorbance of the resulting indicator solution is analyzed according to a standardization curve that directly relates absorbance to organic carbon content. The organic carbon content for the catalyzed samples is corrected through comparison with a simple hydrolysis of cellulose and water done at the same reaction temperature. Further analysis compares organic carbon content to the amount of catalyst used based on utilization of the unit ppt/mole.

3.3 Instruments

The completion of the procedure requires two main instruments. The first of which, the HACH DRB 200 is a digester machine that is the central instrument for both the hydrolysis and TOC procedures. The second, a Spectrovis spectrophotometer is used to measure absorbances of the samples. The absorbance spectrum is collected and analyzed using the computer software program LoggerLite.

4. Results

To begin conducting cellulose hydrolysis trials, the first step is calibration of the HACH DRB 200 instrument for total organic carbon (TOC) analysis. The procedure followed that which is outlined in the document posted on HACH's support website. [27] The one deviation from the manufacturers procedure is that our experimental procedure utilizes UV-Vis absorbance spectrometry to analyze organic carbon. In this event, five concentration standards of 4, 6, 8, 10, and 20 ppm KHP were selected to serve as standards in construction of a calibration curve of absorbances. UV-vis measurements analyzed the absorbances of the resulting colorimetric ampules. It was experimentally found that the solutions contained a λ_{max} equal to 591.8 nm.

Figure 5 and Table 1, below, serves as an example of the obtained absorbance spectrums, and a summary of the KHP absorbance results, respectively.
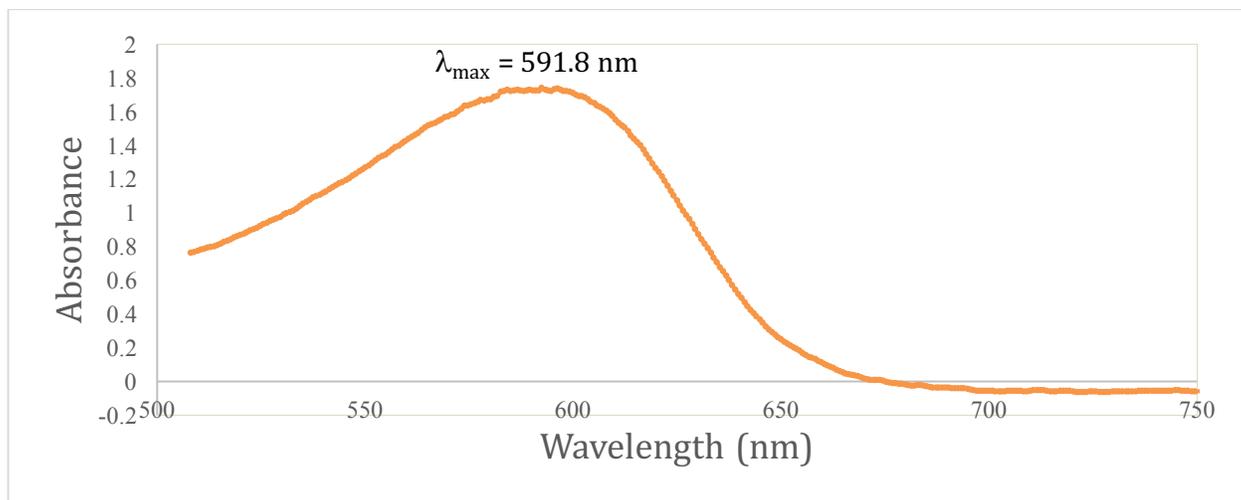
Figure 5. Example absorbance spectrum obtained through uv-vis analysis of the individual ampules.

Table 1. Absorbance Data of KHP Standards

KHP Standard	Absorbance
Blank	1.5523
4 ppm	1.5003
6 ppm	1.3610
8 ppm	1.2627
10 ppm	1.1657
20 ppm	0.4400

The calibration curve is directly applied for absorbances of total organic carbon analyses in order to reveal the amount of organic carbon present after cellulose hydrolysis. Figure 6 demonstrates the obtained calibration curve for the KHP standards:
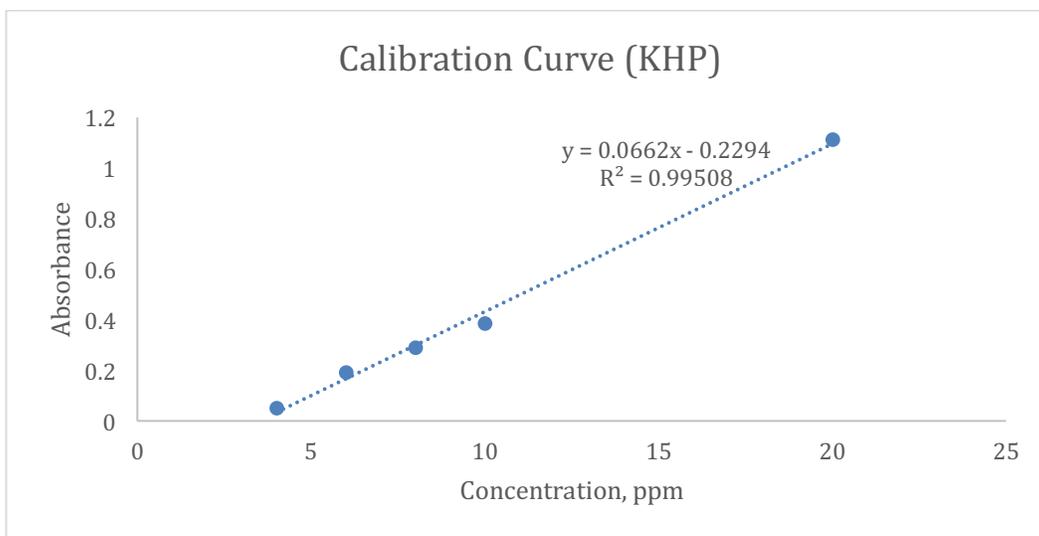
Figure 6: Calibration curve for the 5 KHP standards at 4, 6, 8, 10, and 20 ppm.

The application of a trendline provides the necessary equation to calculate the concentration of organic carbon based on absorbance. The correlation coefficient produced by the trendline is equal to 0.995, which is strong enough to conclude a linear relationship.

Upon proper instrument calibration, hydrolysis trials of cellulose in acids were performed. In order to obtain an average of the results and assess the precision of the hydrolysis, triplicate samples were run per each experimental trial. Both the PMA catalyst and microcrystalline cellulose reagents were used as purchased from Sigma Aldrich, Phosphomolybdic Acid Hydrate, A.C.S. Reagent (CAS 61429-74-4) and Spectrum Chemical, Microcrystalline Cellulose (CAS 9004-34-6), respectively. Sample solutions for hydrolysis were prepared according to the following parameters:

Table 2. Parameters continuously used in the experimental hydrolysis trials

Sample	Contents
Cellulose and Water (blank)	0.1 g microcrystalline cellulose, 4 mL DI water
Cellulose, Water, and Phosophomolybdic acid	0.1 g microcrystalline cellulose, 4.0 mL DI water, 1.86 g solid phosphomolybdic acid

The contents were placed in small vials that were capped and placed in the HACH DRB 200 for digestion at varying temperatures for two hours. After two hours, the hydrolysis samples were removed and allowed to cool at room temperature until they are safe to handle. Contents of the digested samples were then emptied into a syringe attached to nano-filters (50 μm). In theory, filtration halts hydrolysis processes by permitting the passage of hydrolyzed glucose products, but removing polymers of cellulose. The hydrolysis vial was rinsed with deionized water to collect all products. Upon completion, the filtrate is diluted to a total of 200 mL with deionized water using a volumetric flask.

The diluted hydrolysis products were then subject to TOC analysis. As described previously, the procedure for TOC analysis followed the one outlined in the manufacturers document and as described in the TOC calibration procedure. [27]

Absorbance values obtained after TOC analysis were compared against the concentration calibration curve established previously. Calculations for the determination of organic carbon content according to the calibration curve reflected absorbance values of the hydrolysis trials that were subtracted from the absorbance of cellulose and water sample in order to effectively describe the impact of the catalyst on the system. Additionally, in the calculation of organic carbon concentrations following experimental hydrolysis trials is the inclusion of a dilution factor equal to 50 stemming from the dilution of the hydrolyzed products from 4 mL to 200 mL. Table 3, below, summarizes the results of the hydrolysis temperature trials at 40, 60, 80, and 100 °C, and Figure 7 provides a graphical representation of the trials in units of parts per thousand/mole (ppt/mol). Note that the ppm/mol amounts were found by subtracting the blank composed of cellulose and water.

Table 3. Summarizes the results of the different trials at systematic temperature trials.

| Sample	Avg. Absorbance	Avg. ppt	ppt/mol
40 °C trials			
Cellulose + water	1.549	.0269	-
Phosphomolybdic Acid (PMA) catalyst	1.587	.1446	138.8
60 °C trials			
Cellulose + water	1.682	.0288	-
Phosphomolybdic Acid (PMA) catalyst	1.63	.2089	200.8
80 °C trials			
Cellulose + water	1.735	.0297	-
Phosphomolybdic Acid (PMA) catalyst	1.563	.3037	292.3
100 °C trials			
Cellulose + water	1.636	.0282	
Phosphomolybdic Acid (PMA) catalyst	1.081	.5924	571.7
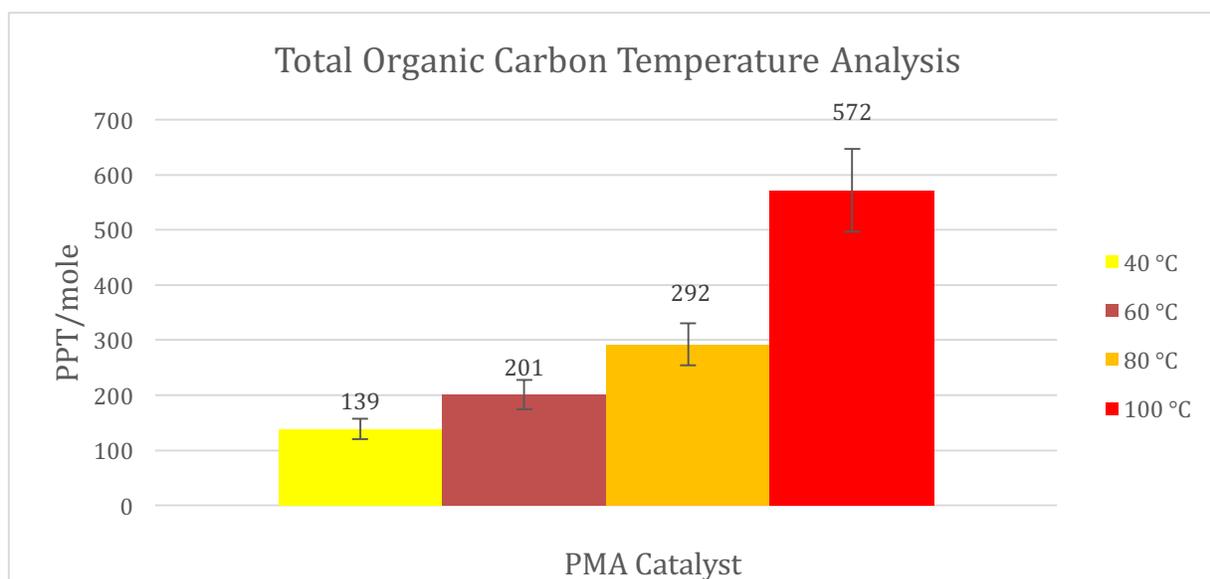
Figure 7: Graphical representation of the PMA results seen in Table 3.

Ultimately, as demonstrated in the cellulose and water blanks, simple hydrolysis demonstrates consistently small total organic carbon concentrations across all temperatures. This result speaks to the stability of the cellulose structure. PMA catalyzed hydrolysis trials, however,

demonstrate that catalysis of cellulose to hydrolyzed products is promising. Additionally, PMA catalyzed hydrolysis trials fulfill the hypothesis that the PMA demonstrates thermoactivation, evidenced by the fact that catalyzed hydrolysis follows a clear pattern of increased organic carbon content with increasing temperatures. The difference in trials of 80 °C to 100 °C demonstrates almost exponential behavior as there was significant 96% upsurge in organic carbon content. Although the liquid boiling point of aqueous PMA solution has not been observed in our lab, it is proposed that the interaction of boiling water molecules in the 100 °C hydrolysis system positively influenced hydrolysis efficiency. Given that microcrystalline cellulose demonstrates a melting point greater than 260 °C, the possibility that the 100 °C system was enough to induce conformational changes in the cellulose structure can be disregarded.

Efforts were taken to minimize error in experimental methods and analysis. The error bars shown in Figure 7 convey an uncertainty equal to 13.2 %. This uncertainty is a product of the uncertainty in the y intercept value of the calibration curve. Uncertainty in the glassware and instruments used throughout the procedure also made minimal contributions to the overall uncertainty value. When calculating the results, efforts were taken to minimize the presence of confounding variables in the form of organic carbon content that could have possibly resulted from simple hydrolysis of cellulose with water. This was accomplished via subtraction of the absorbance of the catalyzed hydrolysis from the absorbance from the cellulose and water hydrolysis.

5. Future Work:

While much work has been invested in this study, this project remains incomplete. Considerations for the future include glucose-specific detection, evaluation of catalyst recyclability, and investigation of the use of PMA to power fuel cells. First, as mentioned earlier,

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glucose-specific tests are both complicated and energy-intensive. For these reasons, TOC was selected as the initial analysis method in order to test the hypothesis while still minimizing the investment in the project. Now that early results indicate the potential of PMA as a catalyst, more information must be obtained through glucose-specific tests such as the chromatographic techniques used in literature.[28] As mentioned previously, chemical hydrolysis methods are prone to yield unintended byproducts. A glucose-specific detection would provide more information about glucose concentration as well as the selectivity of the catalyst to yield the intended products. Next, investigation into the recyclability of the catalyst is necessary. Catalysts employed in industrial production are preferred to demonstrate efficacy throughout continued usage. Therefore, it will be important to employ catalyst separation methods, such as distillation methods used in literature.[7, 20] Results of the hydrolysis trials with repeated use of the PMA catalyst may then be evaluated. One especially important consideration will be the recyclability of the catalyst after hydrolysis trials of temperatures equal to or greater than 100 °C as boiling water could possibly induce catalyst decomposition. The last avenue for important future research concerns the use of PMA solution to power fuel cells. Specifically, solid state microbial fuel cells are an intriguing application in which organic material is used to feed a cell that drives an electric current by using bacteria.

6. **Conclusions:**

Our unsustainable dependence on fossil fuels to provide energy for a growing industrialized world motivates the research of alternative energy sources. In a field that includes solar, wind, hydro and other emerging energy sources, investigation into cellulosic derived biofuels is important for development and optimization. Cellulosic materials are an especially attractive option for implementation in biofuels abundant, economical, and unlike corn starch is

of little use to human dietary consumption. While the energy content of cellulose in nature holds potential to sufficiently supply the worlds energy demands, improvements upon current methods of cellulosic biofuel production are first required. Of the phases of production that needs improvement, the hydrolysis step takes priority. Although current biochemical enzymatic hydrolysis operates under mild conditions and is a selective process, enzymatic pathways continually remain hindered by availability of the enzymes and costs. Therefore, improvements in chemical methods of hydrolysis hold the most potential to the advancement of cellulose derived biofuels. Whereas current industrial methods utilize mineral acids under harsh conditions, the proposal to use a heteropoly acid operated under mild conditions such as that of the PMA catalyzed methods used herein holds early promise to be an efficient replacement. Our investigation revealed that PMA demonstrates thermoactivation with the greatest organic carbon yield equal to 572 ppt/mole of catalyst corresponds to the highest hydrolysis temperature of 100 °C; a 96% improvement from the hydrolysis trial performed at 80 °C. While thermoactivation was anticipated, this study provides an energy-conscious framework into the minimal energy requirements (system temperature) necessary to yield positive results. By investigating this relationship maximization of net energy gains is possible through optimization of the procedure. It is therefore the hope of the experimenters that this study provides insight into continued investigation of PMA as a catalyst in the hydrolysis of cellulose. Future experimentation will require the measurement of specific sugar amounts, and the ability of PMA to be recycled and continually used as an efficient catalyst.

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